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PATENTS

Attorney Docket No. VPI/97-104 CON

Applicant(s) : Michael S. Su et al.

For : METHODS FOR DESIGNING INHIBITORS OF SERINE/THREONINE KINASES
AND TYROSINE KINASES

EXPRESS MAIL CERTIFICATION

"Express Mail" mailing label number EH623260566US
Date of Deposit August 18, 2000

I hereby certify that this transmittal letter and the other papers and fees identified in this transmittal letter as being transmitted herewith being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. § 1.10 on the date indicated above and are addressed to the Hon. Assistant Commissioner for Patents, Washington, D.C. 20231.

Hon. Assistant Commissioner
for Patents
Washington, D.C. 20231

TRANSMITTAL LETTER FOR RULE 53(b)
CONTINUING PATENT APPLICATION

Sir:

This is a request for filing a ☒ continuation, ☐ divisional, application of pending prior Application No. PCT/US99/03181, filed February 16, 1999.

Transmitted herewith for filing are the ☒ specification; ☒ claims; ☒ abstract; ☒ declaration; ☒ power of attorney; ☒ Statements Under 37 C.F.R. § 1.821 for the above-identified patent application.

The enclosed declaration is:

- ☒ Unexecuted (original or copy).
- ☐ Copy from a prior application (37 C.F.R. § 1.63(d)).
- ☐ A signed statement is attached deleting inventors named in the prior application (37 C.F.R. §§ 1.63(d)(2) and 1.33(b)).
- ☒ The entire disclosure of the prior application, from which a copy of the declaration is supplied, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

☐ The prior application, Application No. _____, filed _____, is assigned of record to _____.

Also transmitted herewith are:

☐ _____ sheets of:



08/18/00 08/18/00



☐ Formal drawings.

☐ Informal drawings. Formal drawings will be filed during the pendency of this application.

☒ Preliminary Amendment (5 pp.)

☐ An assignment of the invention to _____

☐ A check in the amount of \$40.00 to cover the recording fee.

☐ Please charge \$40.00 to Deposit Account No. 06-1075 in payment of the recording fee. A duplicate copy of this transmittal letter is transmitted herewith.

☐ An associate power of attorney.

☐ A certified copy of the priority document, _____ application, No. _____, filed _____.

☒ A verified statement to establish small entity status under 37 C.F.R. §§ 1.9 and 1.27 ☒ is enclosed, ☐ was filed in the prior application and such status is still proper and desired (37 C.F.R. § 1.28(a)).

The filing fee has been calculated as shown below:

FOR	NUMBER FILED	NUMBER EXTRA	RATE	FEE
BASIC FEE				\$ 345.00
TOTAL CLAIMS	12	- 20 = 0	x \$ 09	= \$
INDEPENDENT CLAIMS	1	- 3 = 0	x \$ 39	= \$
<input checked="" type="checkbox"/> A MULTIPLE DEPENDENT CLAIM			+ \$130	= \$ 130.00
TOTAL				\$ 475.00

☒ A check in the amount of \$ 475.00 in payment of the filing fee is transmitted herewith.

☒ The Commissioner is hereby authorized to charge payment of any additional filing fees required under 37 C.F.R. § 1.16 in connection with the paper(s) transmitted herewith, or credit any overpayment of same, to Deposit Account No. 06-1075. A duplicate copy of this transmittal letter is transmitted herewith.

☐ Please charge \$ _____ to Deposit Account No. 06-1075 in payment of the filing fee. A duplicate copy of this transmittal letter is transmitted herewith.

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STATEMENT CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) & 1.27(c))—SMALL BUSINESS CONCERNDocket Number (Optional)
VPI/97-104Applicant, Patentee, or Identifier: Michael S. Su et al.
Application or Patent No.: Filed herewith
Filed or Issued: herewith
Title: METHODS FOR DESIGNING INHIBITORS OF SERINE/THREONINE KINASES AND
TYROSINE KINASES

I hereby state that I am

- ☐ the owner of the small business concern identified below:
☒ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF SMALL BUSINESS CONCERN Vertex Pharmaceuticals IncorporatedADDRESS OF SMALL BUSINESS CONCERN 130 Waverly Street, Cambridge, Massachusetts
02139

I hereby state that the above identified small business concern qualifies as a small business concern as defined in 13 CFR Part 121 for purposes of paying reduced fees to the United States Patent and Trademark Office, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time, or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control it.

I hereby state that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention described in:

- ☒ the specification filed herewith with title as listed above.
☐ the application identified above.
☐ the patent identified above.

If the rights held by the above identified small business concern are not exclusive, each individual, concern, or organization having rights in the invention must file separate statements as to their status as small entities, and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e).

Each person, concern, or organization having any rights in the invention is listed below:

- ☒ no such person, concern, or organization exists.
☐ each such person, concern, or organization is listed below.

Separate statements are required from each named person, concern or organization having rights to the invention stating their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b))

NAME OF PERSON SIGNING Richard AldrichTITLE OF PERSON IF OTHER THAN OWNER Senior Vice President/Chief Business OfficerADDRESS OF PERSON SIGNING 130 Waverly Street, Cambridge, MA 02139SIGNATURE  DATE Feb 17, 1998

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APPLICATION INFORMATION

Title Line One:: METHODS FOR DESIGNING INHIBITORS OF SERI
Title Line Two:: NE/THREONINE KINASES AND TYROSINE KINASE
Title Line Three:: S
Total Drawing Sheets:: 0
Formal Drawings?: No
Application Type:: Utility
Docket Number:: VPI97-104CON
Secrecy Order in Parent Appl.?: No

REPRESENTATIVE INFORMATION

Representative Customer Number:: 1473
Registration Number One:: 27794
Registration Number Two:: 33259
Registration Number Three:: 43866

CONTINUITY INFORMATION

This application is a:: CONTINUATION OF
> Application One:: PCT/US99/03181
Filing Date:: 02-16-1999

Which is a:: CONTINUATION OF
>> Application Two:: 09/025580
Filing Date:: 02-18-1998

Source:: PrintEFS Version 1.0.1

VPI/97-104 CON

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner : Not yet assigned
Group : Not yet assigned
Applicants : Michael S. Su et al.
Serial No. : Not yet assigned
Filed : Concurrently herewith
For : METHODS FOR DESIGNING INHIBITORS OF
SERINE/THREONINE KINASES AND TYROSINE
KINASES

New York, New York
August 18, 2000

Hon. Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Prior to examining this application, please amend the application as follows:

IN THE SPECIFICATION

On page 1, before "TECHNICAL FIELD OF THE INVENTION,"
please add the following:

--This is a continuation of PCT/US99/03181, filed February 16, 1999,
which in turn claims benefit from United States application 09/025,580, filed February
18, 1998.--

IN THE CLAIMS

Please cancel claims 1-9 before calculating the filing fee.

Please amend claims 10-16 as follows:

10. (Amended) A mutant of a naturally occurring second serine/threonine protein kinase or tyrosine protein kinase, said mutant characterized by:

a. having an ATP binding site comprising at least one amino acid substitution [in an ATP binding site as] compared to an ATP binding site of the [a corresponding] naturally occurring second serine/threonine protein kinase or tyrosine protein kinase; and

b. having the ability to bind to a compound that binds to an ATP binding site of a first serine/threonine protein kinase or first tyrosine protein kinase.
said binding of the compound with the mutant having [with] a K_i or a K_d that is

(i) [of] less than 10 μ M [a compound that binds to an ATP binding site of a first serine/threonine kinase or tyrosine kinase;] and

c. (ii) [the ability to bind said compound with] at least [a] 10-fold lower [K_i or K_d] than the K_i or K_d [for] of the binding of said compound with said naturally-occurring second serine/threonine protein kinase or second tyrosine protein kinase.

11. (Amended) The mutant second protein kinase according to claim 10, wherein said first and said second protein kinases are mitogen activating protein (MAP) kinases.

12. (Amended) The mutant second protein kinase according to claim 11, wherein said mutant second protein kinase is selected from:

a. a mutant extracellular-signal regulated kinase 2 (ERK2) [consisting of] comprising the amino acid sequence [as set forth in] of SEQ ID NO:2, wherein amino acid 105 is threonine or alanine; or

b. a mutant Jun-N-terminal kinase 3 (JNK3) comprising amino acids 40-402 of SEQ ID NO:3, wherein amino acid 146 is threonine or alanine.

13. (Amended) The mutant ERK2 [second kinase] according to claim 12, wherein [in SEQ ID NO:2] amino acid 103 is leucine, amino acid 106 is histidine, amino acid 109 is glycine and amino acid 110 is alanine.

14. (Amended) The mutant JNK3 [second kinase] according to claim 12, wherein [in SEQ ID NO:3] amino acid 150 is glycine.

15. (Amended) A crystallizable co-complex of a mutant protein second kinase according to any one of claims 10 to 14 and an inhibitor of said first kinase bound to the ATP binding site of said mutant second protein kinase.

16. (Amended) The crystallizable co-complex according to claim 13, wherein said first protein kinase is p38, said second protein kinase is a MAP kinase and said inhibitor is a pyridinyl-imidazole inhibitor of p38.

REMARKS

Applicants have amended claims 10-16 to recite that the serine/threonine and tyrosine kinases are protein kinases. Support for this amendment is found throughout the specification. See, e.g., page 1, line 17. Applicants have further amended claim 10 to clarify that the binding of a compound to the mutant protein kinase is compared to the binding of the compound to the second serine/threonine protein kinase or second tyrosine protein kinase. Support for this amendment is found throughout the specification. See, e.g., page 7, lines 20-24.

Applicants have amended claim 11 to define a MAP kinase as a mitogen activated protein kinase. Applicants have amended claim 12 to define ERK2 and JNK3 as extracellular-signal regulated kinase 2 and Jun N-terminal kinase 3, respectively. Support for these amendments are found, *inter alia*, at page 2, lines 21-22 and 28-29.

Applicants have further amended claims 10 and 12-16 to improve their form.

None of these amendments adds new matter. Their entry is requested.

Respectfully submitted,

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VPI 97-104

METHODS FOR DESIGNING INHIBITORS OF SERINE/THREONINE-
KINASES AND TYROSINE KINASES

TECHNICAL FIELD OF INVENTION

5 The invention relates to methods for designing
inhibitors of serine/threonine kinases, particularly MAP
kinases, and tyrosine kinases through the use of ATP-
binding site mutants of those kinases. The methods of
this invention take advantage of the fact that the mutant
10 kinases are capable of binding inhibitory compounds of
other kinases with greater affinity than the
corresponding wild-type kinase. The invention further
relates to the mutant kinases themselves and
crystallizable co-complexes of the mutant kinase and the
15 inhibitory compound.

BACKGROUND OF THE INVENTION

Kinases and protein kinase cascades are
involved in most cell signaling pathways, and many of
these pathways play a role in human disease. For
20 instance, kinases have been implicated in cell entry into
apoptosis [P. Anderson, Micobiol. Mol. Biol. Rev., 61,
pp. 33-46 (1997)], cancer [P. Dirks, Neurosurgery, 40,
pp. 1000-13, (1997)], Alzheimer's disease [K. Imahori et
al., J. Biochem., 121, pp. 179-88 (1997)] angiotensin II
25 and hematopoietic cytokine receptor signal transduction
[B. Berk et al., Circ. Res., 80:5, pp. 607-16 (1997); R.
Mufson, FASEB J., 11:1 pp. 37-44 (1997)], oncoprotein
signaling and mitosis [A. Laird et al., Cell Signal, 9:3-
4 pp. 249-55 (1997)], inflammation and infection [J. Han
30 et al., Nature, 386 296-9 (1997).] An understanding of

the structure, function, and inhibition of kinase activity could lead to useful human therapeutics.

The structures of a number of protein kinases have been solved by X-ray diffraction and analyzed [reviewed in L. Johnson et al., Cell, 85, pp. 149-158 (1996); E. Goldsmith et al., Cur. Opin. Struct. Biol., 4, pp. 833-840 (1994); S. Taylor et al., Structure, 2, pp. 345-355 (1994)]. Enzymes in the kinase family are often characterized by two domains separated by a deep channel. The N-terminal domain creates a binding pocket for the adenine ring of ATP, and the C-terminal domain contains the presumed catalytic base, magnesium binding sites, and phosphorylation lip. Sequence homology among the kinases varies, but is usually highest in the ATP-binding site. ATP is a substrate common for all kinases.

Among medically important tyrosine kinases are epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), fibroblast growth factor receptor (FGFR), Flk-1, and src.

One particularly important class of serine/threonine kinases are the mammalian mitogen-activated protein (MAP)1 kinases. These kinases mediate intracellular signal transduction pathways [M. H. Cobb et al., J. Biol. Chem., 270, pp. 14843-6 (1995); R. J. Davis, Mol. Reprod. Dev., 42, pp. 459-67 (1995)]. Members of the MAP kinase family share sequence similarity and conserved structural domains, and include the extracellular-signal regulated kinases (ERKs), Jun N-terminal kinases (JNKs) and p38 kinases. JNK and p38 kinases are activated in response to the pro-inflammatory cytokines TNF- α and interleukin-1, and by cellular stress such as heat shock, hyperosmolarity, ultraviolet

radiation, lipopolysaccharides and inhibitors of protein synthesis [B. Derijard et al., Cell, 76, pp. 1025-37 (1994); J. Han et al., Science, 265, pp. 808-11 (1994); J. Raingeaud et al., J. Biol. Chem., 270, pp. 7420-6
5 (1995); L. Shapiro et al., Proc. Natl. Acad. Sci. U.S.A., 92, pp. 12230-4 (1995)]. In contrast, ERK kinases are activated by mitogens and growth factors [D. Bokemeyer et al., Kidney Int., 49, pp. 1187-98 (1996)].

ERK2 is found in many different cell types.

10 ERK2 is a protein kinase that achieves maximum activity when both Thr183 and Tyr185 are phosphorylated by the upstream MAP kinase kinase, MEK1 [N. G. Anderson et al., Nature, 343, pp. 651-3 (1990); C. M. Crews et al., Science, 258, pp. 478-80 (1992)]. Upon activation, ERK2
15 phosphorylates many regulatory proteins, including the protein kinases Rsk90 [C. Bjorbaek et al., J. Biol. Chem. 270, pp. 18848-52 (1995)] and MAPKAP2 [J. Rouse et al., Cell, 78, pp. 1027-37 (1994)], and transcription factors such as ATF2 [J. Raingeaud et al., Mol. Cell. Biol., 16,
20 pp. 1247-55 (1996)], Elk-1 [J. Raingeaud et al. (1996)], c-Fos [R. H. Chen et al., Proc. Natl. Acad. Sci. U.S.A., 90, pp. 10952-6 (1993)], and c-Myc [B. L. Oliver et al., Proc. Soc. Exp. Biol. Med., 210, pp. 162-70 (1995)].

ERK2 is also a downstream target of the Ras/Raf dependent
25 pathways [S. A. Moodie et al., Science, 260, pp. 1658-61 (1993)] and may help relay the signals from these potentially oncogenic proteins. ERK2 has been shown to play a role in the negative growth control of breast cancer cells [R. S. Frey et al., Cancer Res., 57, pp.
30 628-33 (1997)] and hyperexpression of ERK2 in human breast cancer has been reported [V. S. Sivaraman et al., J. Clin. Invest., 99, pp. 1478-83 (1997)]. Activated

ERK2 has also been implicated in the proliferation of endothelin-stimulated airway smooth muscle cells, suggesting a role for this kinase in asthma [A. Whelchel et al., Am. J. Respir. Cell. Mol. Biol., 16, pp. 589-96 (1997)]. In addition, ERK2 appears to be involved in platelet-derived growth factor-directed migration of vascular smooth muscle cells, suggesting that this kinase may be also be involved in restenosis and hypertension. [K. Graf et al., Hypertension, 29:1, pp. 334-339 (1997)].

10 The crystal structures of unphosphorylated p38 [K. P. Wilson et al., J. Biol. Chem., 271, pp. 27696-700 (1996); Z. Wang et al., Proc. Natl. Acad. Sci. U.S.A., 94, pp. 2327-32 (1997); (Brookhaven PDB entry, 1WFC)], and ERK2 [F. Zhang et al., Nature, 367, pp. 704-11 (1994); 15 (Brookhaven PDB entry, 1ERK)] have been solved. Recently, a phosphorylated ERK2 crystal structure has also been solved [B. J. Canagarajah et al., Cell, 90, pp. 859-69 (1997)]. The fold and topology of ERK2 is similar to p38 [K. P. Wilson et al. (1996)], and the two proteins 20 are 48% identical in amino acid sequence.

 p38 was identified as a kinase that was phosphorylated on tyrosine following stimulation of monocytes by LPS [J. C. Lee et al., Nature, 372, pp. 739-46 (1994)]. p38 kinase was cloned [J. Han et al. (1994)] 25 and shown to be the target for pyridinylimidazole compounds that block the production of IL-1 β and TNF- α by monocytes stimulated with LPS [J. C. Lee et al. (1994)]. SB203580, a 2,4,5-triarylimidazole, is a potent p38 kinase inhibitor that is selective relative to other 30 kinases, including other closely related MAP kinases [A. Cuenda et al., FEBS Lett., 364, pp. 229-33 (1995); A. Cuenda et al., EMBO J., 16, pp. 295-305 (1997)]. The

structure of SB203580 in complex with p38 has been reported [L. Tong et al., Nat. Struct. Biol., 4, pp. 311-6 (1997)]. The crystal structure of a different pyridinylimidazole compound, VK-19,911, 4-(4-fluorophenyl)-1-(4-piperidinyl)-5-(4-pyridyl)-imidazole in complex with p38 has also been described [K. P. Wilson et al., Chem. & Biol., 4, pp. 223-231 (1997)]. These structures identified the residues important for binding pyridinyl-imidazoles, and revealed that both compounds bind within the ATP binding site of p38. Many of these residues are conserved in ERK2, but there are enough differences that binding of pyridinyl-imidazole compounds does not occur. A similar situation exists for JNK3, which also shares structural similarity to p38, but is unable to bind pyridinyl-imidazole inhibitors. This same type of scenario, wherein a compound binds to one family member, but not to the majority of others, is also likely to occur in other serine/threonine kinase and tyrosine kinase families.

However, the kinase family members that do not share affinity for a compound that binds to one member may be equally, if not more important from a medical standpoint. Thus, there is an ongoing need to identify potential inhibitors of those other kinases.

SUMMARY OF THE INVENTION

The present invention solves the problem indicated above by providing a method of identifying potential inhibitors of serine/threonine kinases and tyrosine kinases that are related to a kinase which has a known inhibitor. In particular, the invention provides a method of identifying potential inhibitors of ERK2 and

JNK3, as well as other MAP kinases that are unable to bind pyridinylimidazole compounds which inhibit the MAP kinase p38.

5 The method of the present invention is based upon the identification of residues in the ATP-binding pocket of a first kinase that make close contacts with an inhibitor. This may be achieved by crystallizing a first kinase with a known inhibitor and analyzing the data. Alternatively, such data may already be available.

10 Once this information is provided, related kinases are identified using readily available protein alignment software and databases of proteins. Related kinases which share some, but not all, of the first kinase ATP binding pocket amino acid residues that
15 interact with the known inhibitor are selected as candidates for which new inhibitors may be designed.

 One or more of the amino acid residues in the ATP binding pocket of the related ("second") kinase which could potentially interact with the known inhibitor, but
20 which are different from the corresponding amino acid residue in the first kinase are then altered to increase affinity for the known inhibitor. This "mutated" or "mutant" second kinase is also part of the present invention. The ability of the known inhibitor to bind to
25 the mutant second kinase with good affinity is confirmed by binding studies.

 Once affinity is confirmed, the mutant second kinase-known inhibitor complex is subjected to molecular modeling means (X-ray crystallography, 3-D computer
30 analysis) to determine how to alter the known inhibitor to create a compound which inhibits the wild type second kinase.

The crystallizable co-complex of the mutant second kinase with the known inhibitor is also a part of this invention.

DETAILED DESCRIPTION OF THE INVENTION

5 According to one embodiment, the invention provides a method for designing an inhibitor of a second serine/threonine kinase or a second tyrosine kinase. This method comprises the steps of:

- 10 a. identifying amino acids in an ATP binding site of a first serine/threonine kinase or a first tyrosine kinase which form close contacts with a compound bound to said ATP binding site;
- 15 b. employing protein alignment means to identify a second serine/threonine kinase or a second tyrosine kinase that form some, but not all, of the close contacts formed between said compound and said first serine/threonine kinase or said first tyrosine kinase;
- 20 c. altering an amino acid in the ATP binding site of said second serine/threonine kinase or said second tyrosine kinase to create a mutant second serine/threonine kinase or a mutant second tyrosine kinase, wherein said compound binds with at least 10-fold greater affinity to said mutant second kinase than to said second kinase;
- 25 d. confirming that said compound binds with greater affinity to said mutant second serine/threonine kinase or said mutant second tyrosine kinase than to said second serine/threonine kinase or said second tyrosine kinase; and
- 30 e. using molecular modeling means to

modify said compound to create an inhibitor of said second kinase, such that said inhibitor binds to said second kinase with at least 10-fold greater affinity than said compound binds to said second kinase.

5 The identification of the amino acids in an ATP binding site of a first serine/threonine kinase or a first tyrosine kinase which form close contacts with a compound bound to said ATP binding site is routinely performed by analyzing the X-ray crystal structure of the
10 first kinase co-complexed with an inhibitor that is known to bind to its ATP binding site, or co-complexed with ATP itself.

Standard X-ray crystallographic techniques, equipment and software are used to generate crystals of
15 the co-complex, carry out the X-ray diffraction, collect and analyze the data. These techniques, equipment and software are well known in the art.

It should be understood, however, that generating the X-ray data is not a required step in the
20 method of this invention. One may begin by having this data (either raw or fully analyzed) in hand from previous experiments or from an outside source. One may also begin by acquiring the knowledge of which amino acids make close contact with the bound inhibitor or ATP
25 directly from another source.

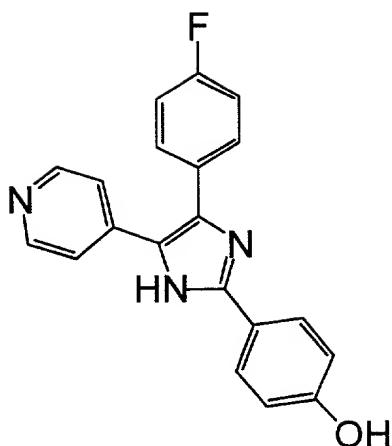
The term "close contact", as used herein, means that an atom or atoms of the ATP binding site of the kinase are physically close enough to an atom or atoms of the compound bound to that site and that the atoms are of
30 such a nature as to enable the formation of non-covalent bonds, such as hydrogen bonds or van der Waals or electrostatic interactions. Physical distances of

less than 4Å are required to form significant non-covalent interactions. A close contact also includes any covalent interactions between the kinase and the ligand.

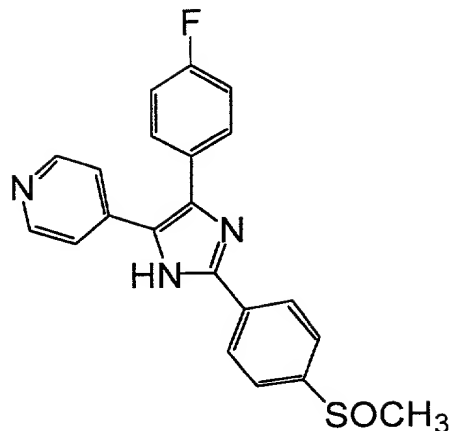
The choice of inhibitor to bind to the kinase
5 in order to generate information on close contacts depends upon the nature of the kinase. The inhibitor should bind tightly to the kinase and significantly inhibit the ability of the kinase to hydrolyze ATP. Any known inhibitor that has a K_d and/or a K_i of less than 1
10 μ M will suffice. Preferably, the inhibitor will have a K_d and/or a K_i of less than 100 nM.

The measurements of K_i for enzyme inhibition and K_d for binding of a ligand to a protein of interest are well known in the art. These are described, for example,
15 in "Enzyme Structure and Mechanism, Second Edition," Alan Fersht, ed., W. H. Freeman and Company, New York (1985), the disclosure of which is herein incorporated by reference.

According to a preferred embodiment, the first
20 kinase is a MAP kinase. Even more preferred is that the first kinase be p38 having the amino acid sequence set forth in SEQ ID NO:1. Preferably, the inhibitor bound to p38 of SEQ ID NO:1 is a pyridinyl-imidazole compound. More preferably, the pyridinyl-imidazole compound is
25 selected from SB203580 or SB 202190, which have the structures depicted below.



SB 202190



SB 203580

Other pyridinyl-imidazole compounds that may be useful to co-complex with p38 are described in United States patents 5,670,527 and 5,658,903, the disclosures of which are herein incorporated by reference.

Once the close contact amino acids have been identified, the next step is to identify a second serine/threonine kinase or tyrosine kinase that forms some, but not all, of the close contacts formed between the ligand and the first kinase. This is achieved by employing protein alignment means comparing the amino acid sequence of the first kinase with a database containing other kinase amino acid sequences, such as GenBank.

Protein alignment means involve the use of computer software that performs a best fit alignment of a first protein with another, related protein. Several state-of-art computer programs are available for homology comparison and alignment of structure- and sequence-related proteins.

One example of homology alignment program is PILEUP (Genetics Computer Group) which compares multiple sequences of related proteins and nucleotides and

generates an alignment of these sequences for comparison.

PILEUP allows one to use primary protein sequence similarity and structure similarity as parameters to set up an alignment of multiple proteins.

5 Once the close contact amino acid residues of first kinase are defined, corresponding residues in the second kinase of interest can be identified from the alignment generated by the program.

10 From a practical consideration, the amino acid residues of the second kinase that align with the close contact amino acids of the first kinase should differ at a least 1 and not more than 4 residues.

Protein alignment means will identify related kinases and the amino acid residues thereof that align
15 with the close contact amino acids of the first kinase and thus could potentially form close contacts with the inhibitor of the first kinase. The amino acids of this second kinase that align with the close contact amino acids of the first kinase, but differ in identity and/or
20 nature therefrom, are the amino acids that will be targeted for replacement in the next step of the method. The term "nature" of an amino acid, as used herein, means its physicochemical characteristics, e.g., polar, non-polar, hydrophobic, hydrophilic, bulky side group, non-
25 bulky side group, acidic, basic, etc.

According to one preferred embodiment, the second kinase is a MAP kinase. Even more preferred is that the second kinase be ERK-2 having the amino acid sequence set forth in SEQ ID NO:2, wherein amino acid 103
30 is isoleucine, amino acid 105 is glutamine, amino acid 106 is aspartic acid, amino acid 109 is glutamic acid and amino acid 110 is threonine; or JNK3 comprising at least

amino acids 40-402 of SEQ ID NO:3, wherein amino acid 146 is methionine and amino acid 150 is aspartic acid.

Those particular amino acids will be changed to be identical to, or at least similar in nature to, the
5 corresponding amino acid in the first kinase to create a mutant second kinase. This alteration will increase the ability of the ligand to bind to the second (now mutant) kinase by at least 10-fold over its affinity for the unmutated second kinase, as measured by K_i or K_d . If the
10 ligand has no detectable binding to the unmutated second kinase (and therefore a 10-fold increase may not be measurable), the ligand should bind to the mutated second kinase with a K_i and/or K_d of less than 10 μ M.

The alteration of one or more amino acid in the
15 ATP binding site of the second kinase according to the next step in the method may be achieved by standard molecular biological means. For example, site-directed mutagenesis, PCR, or other methods of altering the DNA or a cDNA encoding the second kinase is utilized to change
20 an amino acid in that kinase to create a mutant second kinase. Obviously, the mutant kinase will be produced by recombinant DNA means, which are well known in the art.

In one preferred embodiment, the mutant second kinase is an ERK-2 mutant having the amino acid sequence
25 set forth in SEQ ID NO:2, wherein amino acid 105 is threonine or alanine. According to another preferred embodiment, the mutant second kinase is an ERK-2 mutant having the amino acid sequence set forth in SEQ ID NO:2, wherein amino acid 105 is threonine or alanine, amino
30 acid 103 is leucine, amino acid 106 is histidine, amino acid 109 is glycine and amino acid 110 is alanine. In this embodiment, although 5 amino acids have been changed

as compared to naturally occurring ERK-2, only amino acid 105 is considered to be a close contact amino acid. The other altered amino acids were chosen based on proximity to amino acid 105 and because they differed from those present in p38.

In another preferred embodiment, the mutant second kinase is JNK3 mutant kinase comprising amino acids 40-402 of SEQ ID NO:3, wherein amino acid 146 is alanine. According to another preferred embodiment, the mutant second kinase is JNK3 mutant kinase comprising amino acids 40-402 of SEQ ID NO:3, wherein amino acid 146 is alanine and amino acid 150 is glycine.

Once the mutant second kinase has been created at the DNA level and expressed in an appropriate host cell and isolated, the next step of the method of this invention is to confirm its ability to bind to the ligand of the first kinase. This may be achieved by various methods well known in the art for determining K_i and/or K_d .

The step following confirmation of binding between the ligand and the mutant second kinase is the modification of the ligand so that is capable of binding to and inhibiting the ATP binding site of the native form of the second kinase. This step is achieved using molecular modeling means that typically involve solving the crystal structure of the mutant second kinase/ligand co-complex; analyzing the contacts made between the co-complex components; comparing how the ligand would interact with the native second kinase using computer simulation and the appropriate software; and altering those portions of the ligand that are sterically hindered from or otherwise incompatible with binding to the native

second kinase. The software typically utilized in molecular modeling is capable of achieving each of these steps, as well as suggesting potential replacements for various moieties of the ligand that would increase
5 association with the native second kinase.

One skilled in the art may use one of several methods to screen chemical moieties to replace portions of the ligand so that binding to the native second kinase is optimized. This process may begin by side-by-side
10 visual inspection of, for example, native second kinase and the mutant second kinase ATP binding sites on the computer screen based on the X-ray structure of the ligand/mutant second kinase co-complex. Modified ligands may then be tested for their ability to dock to the
15 native second kinase using software such as DOCK and AUTODOCK followed by energy minimization and molecular dynamics with standard molecular mechanics force fields, such as CHARMM and AMBER.

Specialized computer programs may also assist
20 in the process of replacement fragments:

1. GRID (P. J. Goodford, "A Computational Procedure for Determining Energetically Favorable Binding Sites on Biologically Important Macromolecules", J. Med. Chem., 28, pp. 849-857 (1985)). GRID is available from Oxford
25 University, Oxford, UK.

2. MCSS (A. Miranker et al., "Functionality Maps of Binding Sites: A Multiple Copy Simultaneous Search Method." Proteins: Structure, Function and Genetics, 11, pp. 29-34 (1991)). MCSS is available from Molecular
30 Simulations, Burlington, MA.

3. AUTODOCK (D. S. Goodsell et al., "Automated Docking of Substrates to Proteins by Simulated Annealing", Proteins: Structure, Function, and Genetics, 8, pp. 195-202 (1990)). AUTODOCK is available from Scripps Research
35 Institute, La Jolla, CA.

4. DOCK (I. D. Kuntz et al., "A Geometric Approach to Macromolecule-Ligand Interactions", J. Mol. Biol., 151, pp. 269-288 (1982)). DOCK is available from University of California, San Francisco, CA.

5

Alternatively, the portion of the ligand that makes favorable contacts with the identical amino acids in both the mutant and the native second kinase may be retained as a scaffold and used in software programs that
10 create theoretical inhibitors based upon the structure of the native second kinase ATP binding site. These programs include:

1. LUDI (H.-J. Bohm, "The Computer Program LUDI: A New Method for the De Novo Design of Enzyme Inhibitors", J. Comp. Aid. Molec. Design, 6, pp. 61-78 (1992)). LUDI is
15 available from Biosym Technologies, San Diego, CA.

2. LEGEND (Y. Nishibata et al., Tetrahedron, 47, p. 8985 (1991)). LEGEND is available from Molecular Simulations, Burlington, MA.

20 3. LeapFrog (available from Tripos Associates, St. Louis, MO).

Other molecular modeling techniques may also be employed in accordance with this invention. See, e.g., N. C. Cohen et al., "Molecular Modeling Software and
25 Methods for Medicinal Chemistry", J. Med. Chem., 33, pp. 883-894 (1990). See also, M. A. Navia et al., "The Use of Structural Information in Drug Design", Current Opinions in Structural Biology, 2, pp. 202-210 (1992).

Once a compound has been designed or selected
30 by the above methods, the efficiency with which that entity may bind to the native second kinase may be tested and further optimized by computational evaluation.

An entity designed or selected as binding to the native second kinase ATP binding pocket may be

further computationally optimized so that in its bound state it would preferably lack repulsive electrostatic interaction with the target enzyme. Such non-complementary (e.g., electrostatic) interactions include
5 repulsive charge-charge, dipole-dipole and charge-dipole interactions. Specifically, the sum of all electrostatic interactions between the inhibitor and the kinase when the inhibitor is bound to the ATP binding pocket preferably make a neutral or favorable contribution to
10 the enthalpy of binding.

Specific computer software is available in the art to evaluate compound deformation energy and electrostatic interaction. Examples of programs designed for such uses include: Gaussian 92, revision C [M. J.
15 Frisch, Gaussian, Inc., Pittsburgh, PA ©1992]; AMBER, version 4.0 [P.A. Kollman, University of California at San Francisco, ©1994]; QUANTA/CHARMM [Molecular Simulations, Inc., Burlington, MA ©1994]; and Insight II/Discover (Biosym Technologies Inc., San Diego, CA
20 ©1994). These programs may be implemented, for instance, using a Silicon Graphics workstation, Indigo² or IBM RISC/6000 workstation model 550. Other hardware systems and software packages will be known to those skilled in the art.

25 Once the second kinase ATP binding-pocket inhibitory entity has been optimally selected or designed, as described above, substitutions may then be made in some of its atoms or side groups in order to improve or modify its binding properties. Generally,
30 initial substitutions are conservative, i.e., the replacement group will have approximately the same size, shape, hydrophobicity and charge as the original group.

Such substituted chemical compounds may then be analyzed for efficiency of fit to the second kinase ATP binding pocket by the same computer methods described in detail, above.

5 According to another embodiment, the invention provides a mutant second kinase disclosed above. Such a kinase is enzymatically active in its ability to hydrolyze ATP and comprises an amino acid substitution (as compared to the native second kinase) that allows a
10 compound that binds to the ATP binding site of a first serine/threonine kinase or tyrosine kinase to also bind to the ATP binding site of said second serine/threonine or tyrosine kinase. It is preferred that the ATP binding site of the native second kinase, which lacks the amino
15 acid substitution present in the mutant, binds said compound with at least 10-fold lower affinity than said mutant kinase.

 Preferably, the mutant kinase is an ERK-2 kinase having the amino acid sequence of SEQ ID NO:2,
20 wherein amino acid 105 is threonine or alanine; or a mutant JNK3 kinase comprising amino acids 40-402 of SEQ ID NO:3, wherein amino acid 146 is alanine.

 According to another preferred embodiment, both the native second kinase and the first kinase are MAP
25 kinases. More preferred is when the first kinase is p38 having the amino acid sequence of SEQ ID NO:1. Even more preferred is when the native second kinase is ERK-2 having the amino acid sequence of SEQ ID NO:2, wherein amino acid 103 is leucine, amino acid 106 is histidine,
30 amino acid 109 is glycine amino acid 110 is alanine; or JNK3 comprising at least amino acids 40-402 of SEQ ID NO:3, wherein amino acid 146 is methionine and amino acid

150 is aspartic acid. The most preferred of these
embodiments is wherein the compound that binds to the
first kinase and the mutant second kinase is a pyridinyl-
imidazole inhibitor of p38, preferably selected from
5 SB203580 or SB202190.

After aligning the amino acid sequences of ERK2
and p38, we determined that there was a difference in
amino acid type between aligned ERK2 amino acid 105
(glutamine) and p38 amino acid 106 (threonine) (see SEQ
10 ID NOS: 1 and 2). Thus, we changed the ERK2 glutamine
residue to an amino acid with a smaller side group,
preferably threonine or alanine. The resulting mutant
ERK2 enzyme retains its enzymatic activity and can bind a
pyridinyl-imidazole inhibitor of p38.

15 The corresponding amino acids that need to be
altered in other MAP kinases so that they bind pyridinyl-
imidazole compounds with greater affinity can be
identified by aligning its amino acid sequence with that
of ERK2 and/or p38, as discussed above. The amino acid
20 that aligns with amino acid T106 of p38 (SEQ ID NO:1) and
Q105 of ERK2 (SEQ ID NO:2) is the one that will be
targeted for substitution.

The ERK2 mutant containing the above-indicated
amino acid substitution at amino acid 105 plus the
25 following amino acid substitutions: isoleucine-to-leucine
at amino acid 103, aspartic acid-to-histidine at amino
acid 106, glutamic acid-to-glycine at amino acid 109 and
threonine-to-alanine at amino acid 110; maintains its
enzymatic activity, and binds more tightly to pyridinyl-
30 imidazole compounds than the ERK2 with the single
substitution at amino acid 105.

In corresponding fashion, we determined that in

wild-type JNK3, amino acid 146 (methionine) (SEQ ID NO:3) aligned with Thr106 of p38. Thus, we changed the methionine residue to an alanine. The resulting JNK3 mutant retained its enzymatic activity and bound
5 pyridinyl-imidazole compounds with at least 10-fold greater affinity than wild-type JNK3.

In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples
10 are for illustrative purposes only and are not to be construed as limiting this invention in any manner.

EXAMPLE 1

Cloning, Mutagenesis And Expression of Kinases

A. p38

Expression, purification and activation of p38 MAP kinase was as described in K. P. Wilson et al., Chem.
15 & Biol., 4, pp. 223-231 (1997), the disclosure of which is herein incorporated by reference.

B. ERK2

Standard, well-known methods were used for manipulations of recombinant DNA. All subclones were
20 verified by nucleotide sequence analysis of both strands using an Applied Biosystems 373A DNA Autosequencer).

An ERK2 cDNA was cloned by reverse
transcription and subsequent polymerase chain reaction (RT-PCR) of total RNA (Qiagen) prepared from human
25 peripheral lymphocytes (PBLs) which were stimulated with 10 ng/ml phorbol,12-myristate,13-acetate (PMA) and 250 ng/ml ionomycin for 72 hours. The forward primer 5'-GAACGGCGGGCAGCCAACATGGCGGCGGCG-3' (SEQ ID NO:4) and the

reverse primer 5'GGGCTCGAGCCTGACAAATTTAAGATCTGTATCCTG-3'
(SEQ ID NO:5) were used to generate an ERK2 PCR fragment
(RNA PCR kit, Perkin-Elmer) which was cloned into pT7-
Blue (Novagen) to yield pT7-ERK2.

5 For bacterial expression of recombinant ERK2, a
(His)₆ metal affinity tag and a thrombin cleavage site
were introduced at the N-terminus of the translation
product. Simultaneously, *Nde*I and *Bam*HI sites were added
at the 5'- and 3'-end, respectively, by PCR using the
10 forward primer 5'-TTAACATATGGCGGCGGCGGCGGCGGCG-3' (SEQ ID
NO:6) and the reverse primer 5'-CCCACAGGATCCGATCTGTATCCTG
-3' (Perkin-Elmer) (SEQ ID NO:7).

The *Nde*I-*Bam*HI double-digested PCR fragment was
cloned into the appropriate sites of pET-15b (Novagen) to
15 yield pET-ERK2, which was used to transform *E. coli*
BL21(DE3) (Novagen).

Freshly transformed bacteria were grown in LB
broth supplemented with 100 µg/ml carbenicillin at 30°C to
an OD₆₀₀ of 0.7 - 0.9, induced with 1 mM
20 isopropylthiogalactoside (IPTG) for 2 hours, harvested by
low speed centrifugation and stored at -70°C until use.

To facilitate construction of several ERK2
mutants, a silent mutation was introduced into the ERK2
cDNA that provided an additional, single *Hind*III
25 restriction site near the region of mutations. This ERK2
variant (ERK2-HIII) and several ERK2 mutants were
generated by PCR using pT7-ERK2 as template, a forward
primer containing an internal *Sac*II site (underlined),
5'-GATGGTCCGCGGGCAGGTGTTTCG-3' (SEQ ID NO:8) and the
30 following reverse primers containing a *Hind*III site
(underlined) and one or several mutated nucleotides (bold
letters):

(1) for ERK2-HIII 5'-GTGTCTTCAAAAGCTTGTAAAGATCTGTTTCC-3'
(SEQ ID NO:9); (2) for ERK2(Q103T)
5'-CAAAAGCTTGTAAAGATCTGTTTCCATGAGGTCCGTTACTAT-3'; (SEQ ID
NO:10)
5 (3) for ERK2(Q103A)
5'-CAAAAGCTTGTAAAGATCTGTTTCCATGAGGTCCGCTACTAT-3' (SEQ ID
NO:11); and
(4) for ERK2(I101L,Q103T,D104H,E107G,T108A),
5'-CAAAAGCTTGTAAAGATCTGCTCCCATGAGGTGCGTTACTAGATATAC-3'
10 (SEQ ID NO:12). Each of these PCR fragments was digested
with *Sac*II and *Hind*III. Using the forward primer
5'-GATCTTTTACAAAGCTTTTTGAAGACACAAC-3' (SEQ ID NO:13) and
reverse primer 5'-CTTGGTGTAGCCCTTGGAATTCAACATA-3' (SEQ ID
NO:14), a second ERK2 PCR fragment was generated
15 extending from the novel *Hind*III site to an *Msc*I site.
Ligation of the *Sac*II-*Hind*III and *Hind*III-*Msc*I PCR
fragments into *Sac*II-*Msc*I double-digested pT7-ERK2
yielded pT7 subclones for the ERK2-HIII variant and all
ERK2 mutants. These were used to isolate *Sac*II-*Xho*I ERK2
20 cDNA fragments which were ligated into the appropriate
restriction sites of pET-ERK2 for bacterial expression of
(His)₆-tagged recombinant proteins as described above.

C. MEK1

A cDNA encoding a constitutively active mutant
25 of mouse MEK1 (S218D, S222D) [Huang, 1994 #809] with a C-
terminal Glu-Tyr-Met-Pro-Met-Glu (SEQ ID NO:15) tag in
plasmid pG-MEK1Glu was obtained from Dr. R.L. Erikson
(Harvard University, Cambridge, MA). For bacterial
expression of N-terminally (His)₆-tagged (DD)MEK1, two
30 oligodeoxynucleotides 5'-CATGGCACACCATCACCATCACCATCCCAAG
AAGAAGCCGACGCCCATCCAG-3' (SEQ ID NO:16) and 5'-

CTGGATGGGCGTCGGCTTCTTCTTGGGATGGTGATGGTGATGGTGTGC-3' (SEQ
ID NO:17), generating an *Nco*I-*Pvu*II fragment, were
annealed and inserted together with a *Pvu*II-*Bam*HI MEK1
cDNA fragment into *Nco*I-*Bam*HI double-digested pET-
5 BS(+)/T7 to yield pET-BS-(His)₆-MEK1. BL21(DE3) bacteria
were transformed for expression of (His)₆-MEK1 as
described above for ERK2.

D. JNK3

To clone JNK3, standard techniques well-known
10 by those of ordinary skill in the art were used for
manipulations of recombinant DNA.

A BLAST search of the EST database using the
published JNK3α1 cDNA [S. Gupta et al., EMBO J., 15, pp.
2760-70 (1996)] as a query identified an EST clone
15 (#632588, Research Genetics) that contained the entire
coding sequence for human JNK3α1. Polymerase chain
reactions (PCR) using *pfu* polymerase (Stratagene) were
used to introduce restriction sites into the cDNA for
cloning into the pET-15B expression vector at the *Nco*I
20 and *Bam*HI sites for expression of the protein in *E. coli*.
Due to the poor solubility of the expressed full length
protein (Met 1-Gln 422; SEQ ID NO:3), an N-terminally
truncated protein starting at Ser residue at position 40
(Ser 40), corresponding to Ser 2 of JNK 1 and 2 proteins
25 (SEQ ID NOS: 33 and 32), preceded by Met (initiation) and
Gly residues, was produced. The Gly residue was added in
order to introduce an *Nco*I site for cloning into the
expression vector. Further, serial C-terminal truncations
were performed by PCR. This construct, which was
30 prepared by PCR using deoxyoligonucleotides 5'
GCTCTAGAGCTCCATGGGCAGCAAAAGCAAAGTTGACAA 3' (forward
primer with initiation codon underlined) (SEQ ID NO:18)

and 5' TAGCGGATCCTCATTCTGAATTCATTACTTCCTTGTA 3' (reverse primer with stop codon underlined) (SEQ ID NO:19) as primers and confirmed by DNA sequencing, encodes amino acid residues Ser40-Glu402 of JNK3 α 1 (amino acid 40-402 of SEQ ID NO:3), preceded by Met and Gly residues (herein referred to as "tJNK3 α 1").

Site directed mutagenesis of tJNK3 α 1 in the expression vector pET-15B was carried out using the Stratagene^R QuikChangeTM site-directed mutagenesis kit. Oligonucleotides were designed and synthesized to create the tJNK3 α 1 M146A, tJNK3 α 1 M146T and tJNK3 α 1 D150G. The sequence of oligonucleotide pairs used in the mutagenesis were:

- 1) JNK3 M146A - 5' CCA AGA TGT TTA CTT AGT Agc GGA ACT GAT GGA TGC CAA 3' (SEQ ID NO:20) and its complement;
- 2) JNK3 M146T - 5' CAA GAT GTT TAC TTA GTA acG GGA CTG ATG GAT GCC AAC 3' (SEQ ID NO:21) and its complement; and
- 3) JNK3 D150G - 5' GTA ATG GAA CTG ATG GgT GCC AAC TTA TGT CAA GTG 3' (SEQ ID NO:22) and its complement.

Mutant bases are present in lower case. For each mutation, the tJNK3 α 1 pET-15B plasmid was denatured and annealed with the appropriate oligonucleotide pair. PCR reactions were performed using *Pfu*-DNA polymerase to yield nicked circular strands which were digested with *Dpn*I to remove the non-mutated parental DNA template. The resulting material was transformed into XL1-Blue. All mutations were verified by nucleotide sequence analysis using an Applied Biosystems 373A DNA Autosequencer.

For bacterial expression, *E. coli* strain BL21

(DE3) (Novagen) was transformed with tJNK3 α 1, tJNK3 α 1 M146A, tJNK3 α 1 M146T or tJNK3 α 1 D150G. These expression constructs were grown at 30°C in shaker flasks into log phase (OD600 ~ 0.8) in LB supplemented with 100 μ g/ml carbenicillin. IPTG was then added to a final concentration of 0.8 mM and the cells were harvested 2 hours later by centrifugation.

EXAMPLE 2

Purification of MEK1(DD), ERK2, ERK2 Mutants, JNK3 and JNK3 Mutants

10

A. ERK2, ERK2 mutants and MEK1(DD)

Unless otherwise stated all steps were performed at 4°C. *E. coli* cell paste, with expressed kinase, was resuspended in 10 volumes/g lysis buffer (50 mM HEPES, pH 7.8, containing 10% glycerol (v/v), 250 mM NaCl, 5 mM β -ME, 5 mM imidazole, 0.1 mM PMSF, 2 μ g/ml pepstatin, 1 μ g/ml each of E-64 and leupeptin). Cells were mechanically disrupted using a French press and centrifugation at 35,000 x g for 60 min. The supernatant was incubated overnight with 1 ml Talon metal affinity resin (Clontech)/5 - 10 mg estimated protein. Resin with bound kinase was poured into a 1.5 x 10 cm column and washed with 20 column volumes of lysis buffer without protease inhibitors, followed by 20 column volumes of wash buffer (50 mM HEPES, pH 7.5, containing 10% glycerol (v/v), 100 mM NaCl, 5 mM β -ME and 10 mM imidazole).

Protein was eluted in 2-3 column volumes with wash buffer adjusted to pH 8.0 and 100 mM imidazole. 10% precast SDS-PAGE gels (Novex) were used to identify fractions containing MEK1(DD), which were concentrated by ultrafiltration (Centriprep-30, Amicon) to 2 ml.

Concentrated MEK1(DD) was loaded onto a Superdex-75 (60 x 1.6 cm, Pharmacia) column equilibrated with 20 mM HEPES, pH 7.5, containing 10% glycerol (v/v), 100 mM NaCl and 2 mM DTT at a flow rate of 1 ml/min. Eluted MEK1(DD)

5 fractions were stored at -70°C.

All ERK2 kinases were affinity purified as described for MEK1(DD), then diluted to < 25 mM NaCl with 20 mM HEPES, pH 8.0, containing 10% glycerol (v/v) and 2 mM DTT (buffer A), 0.45 μ m filtered, and loaded onto a
10 MonoQ (HR 5/5) anion-exchange column equilibrated in buffer A. After washing with 5% buffer B (buffer A + 1M NaCl), the ERK2 proteins were eluted in a 5-20% buffer B gradient developed over 60 min at 0.5 ml/min and fractions containing ERK2 were stored at -70°C. Protein
15 concentrations were determined from the A₂₈₀ using calculated extinction coefficients of 23,600 and 42,000 M⁻¹ cm⁻¹ for MEK1(DD) and ERK2, respectively.

B. JNK3 and JNK3 Mutants

E. coli cell paste containing JNK3 was
20 resuspended in 10 volumes/g lysis buffer (50 mM HEPES, pH 7.2, containing 10% glycerol (v/v), 100 mM NaCl, 2 mM DTT, 0.1 mM PMSF, 2 μ g/ml Pepstatin, 1 μ g/ml each of E-64 and Leupeptin). Cells were lysed on ice using a microfluidizer and centrifuged at 100,000 x g for 30 min
25 at 4°C. The 100,000 x g supernatant was diluted 1:5 with Buffer A (20 mM HEPES, pH 7.0, 10% glycerol (v/v), 2 mM DTT) and purified by SP-Sepharose (Pharmacia) cation-exchange chromatography (column dimensions: 2.6 x 20 cm) at 4°C. The resin was washed with 5 column volumes of
30 Buffer A, followed by 5 column volumes of Buffer A containing 50 mM NaCl. Bound JNK3 was eluted with a 7.5

column volume linear gradient of 50-300 mM NaCl, where JNK3 eluted between 150-200 mM NaCl.

EXAMPLE 3

In Vitro Phosphorylation of ERK2 and JNK3 proteins

5 ERK2 was diluted to 0.5 mg/ml in 50 mM HEPES, pH 8.0, 10% glycerol, 100 mM NaCl, 2 mM DTT, 20 mM β -glycerophosphate, 10 mM $MgCl_2$. Activation was initiated by addition of 2.5 mM ATP and a 1/25 molar ratio of MEK1(DD) for 1 h at 25°C. Activated ERK2 proteins were
10 diluted to 25 mM NaCl and purified by anion-exchange as described.

The ERK2 mutants are phosphorylated in vitro as efficiently as wild-type enzyme by MEK1.

Five mg of JNK3 was diluted to 0.5 mg/ml in 50
15 mM HEPES buffer, pH 7.5, containing 100 mM NaCl, 5 mM DTT, 20 mM $MgCl_2$, 1 mM ATP. GST-MKK4(DD) kinase (the upstream mutant form of one of the activating kinases of JNK3) was added at a molar ratio of 1 GST-MKK4:2.5 JNK3. After 30 min at 25°C the reaction mixture was
20 concentrated 5-fold by ultrafiltration in a Centriprep-30 (Amicon, Beverly, MA), then diluted back up to 10 ml and an additional 1 mM ATP added. This procedure was repeated three times to remove ADP and replenish ATP. The final (third) addition of ATP was 5 mM and the
25 mixture incubated overnight at 4°C.

The activated JNK3/GST-MKK4(DD) reaction mixture was exchanged into 50 mM HEPES buffer, pH 7.5, containing 5 mM DTT and 5% glycerol (w/v) by dialysis or ultrafiltration. The reaction mixture was adjusted to
30 1.1 M potassium phosphate, pH 7.5, and purified by hydrophobic interactions chromatography (at 25°C) using a

Rainin Hydropore column. GST-MKK4 and unactivated JNK3 do not bind under these conditions and when a 1.1 to 0.05M potassium phosphate gradient is developed over 60 min at a flow rate of 1 ml/min, doubly phosphorylated JNK3 is separated from singly phosphorylated JNK.

Activated JNK3 (i.e. doubly phosphorylated) was stored at -70°C at 0.25-1 mg/ml.

EXAMPLE 4

Kinase Assays

A coupled spectrophotometric assay was used in which ADP generated by ERK2, JNK3 or p38 kinase was converted to ATP by PK with the concomitant production of pyruvate from PEP. LDH reduces pyruvate to lactate with the oxidation of NADH. NADH production was monitored at 340 nm using a microplate reader for 20 min at 30°C. Reactions were in 100 mM HEPES, pH 7.6, 10 mM MgCl₂, and started by addition of 100 µM ATP. PK (100 µg/ml), LDH (50 µg/ml), PEP (2 mM) and NADH (140 µM) were added in large excess. Addition of 200 µM KRELVEPLTPSGEAPNQALLR (SEQ ID NO:23) substrate, corresponding to an EGF receptor peptide [F. A. Gonzalez et al., J. Biol. Chem., 266, pp. 22159-63 (1991)], allowed measurement of kinase activity.

In K_i determinations, E + I was pre-incubated for 15 min at 30°C prior to assay by addition of ATP. Inhibition constants were determined by fitting kinetic data to the Morrison tight-binding equation [J. F. Morrison et al., Adv. Enzymol. Relat. Areas Mol. Biol., 61, pp. 201-301 (1988)] using KineTic (BioKin, 1992). ³²P incorporation into ATF2 (0.1 mg/ml) by 7.5 nM kinase was assayed for 10 min at 30°C in 50 mM HEPES, pH 7, 10 mM MgCl₂ and 2 mM DTT, and visualized by autoradiography.

The kinase activity of the ERK2 mutants are comparable to wild-type enzyme. However, ERK2(Q105T) shows a 640 to 2,500-fold increased binding affinity for the pyridinyl-imidazoles tested (Table I), using a lower
 5 limit of 20 μ M for wild-type ERK2 inhibition. ERK2(Q105A) is even more sensitive to this compound class, exhibiting 1,800 to 25,000-fold increased binding (Table I). Mutation of residues, I103L, D106H, E109G, T110A, in addition to Q105T produced an enzyme (herein
 10 referred to as "ERK2(5X)") most sensitive to the pyridinyl-imidazoles, ranging from 0.76 nM for SB203580 to 0.4 nM for SB202190. The K_i values correspond to a 2,900 to 50,000-fold increase in potency of binding of these compounds. These results indicate that the larger
 15 glutamine side chain at residue 105 accounts for the resistance of ERK2 to pyridinyl-imidazoles.

TABLE 1. K_M for ATP-binding and K_i for pyridinyl-imidazole inhibition of ERK2, ERK2 mutants and p38 kinase.

Enzyme	K_M for ATP (μ M)	Inhibition constants, K_i (nM)	
		SB203580	SB202190
ERK2(wild-type)	76 \pm 14	nil	nil
ERK2(Q105A)	51 \pm 6	1.2 \pm 0.3	0.81 \pm 0.19
ERK2(Q105T)	33 \pm 4	13.0 \pm 3.6	6.8 \pm 0.6
ERK2(5X)	26 \pm 2	0.76 \pm 0.14	0.4 \pm 0.04
p38	260 \pm 30	100 \pm 30	30 \pm 8

20 ¹nil indicates no inhibition at 20 μ M

Due to the different K_M values for the wild-type and mutant JNK3 enzymes we assayed each one with different ATP concentrations:
 25 JNK3(wild-type) ATP = 30 μ M

JNK3 (M105A) ATP = 150 μ M

JNK3 (M105A/D109G) ATP = 600 μ M

Enzyme concentrations in the assay were 5-10 nM. As for ERK2, the kinase phosphate acceptor substrate
5 was the EGF receptor peptide (SEQ ID NO:23) used at 200 μ M. Data analysis to determine K_i values was also as described for ERK2.

Wild-type JNK3 differs from ERK2 in that it is moderately sensitive to SB202190. As seen for ERK2,
10 removal of the side-chain of Met146 in JNK3 (the equivalent to Q105 in ERK2) causes a dramatic increase in sensitivity towards SB202190 (~4,000-fold for the M146A mutant). The double mutant is considerably more sensitive than wild-type, but significantly less than
15 observed for the single mutant. The large increase in K_m for this mutant compared to wild-type suggests that ATP binding is also weaker. However, for other pyridinyl-imidazole compounds tested, the double mutant shows enhanced sensitivity relative to both wild-type and the
20 single mutant enzymes. The results are shown in Table 2, below.

TABLE 2. K_M for ATP-binding and K_i for pyridinyl-imidazole inhibition of JNK3 and JNK3 mutants.

Enzyme	K_M for ATP (μ M)	SB202190 K_i (nM)
JNK3(wild-type)	15	1000
JNK3(M146A)	75	0.23
JNK3 (M146A/D150G)	311	1.5

EXAMPLE 5

Crystallization and Structure Determination
of the ERK2(5X)/SB203580 Complex

Crystals of unphosphorylated ERK2(5X) were grown by vapor diffusion when protein (14 mg/ml in 20 mM Tris, pH 7.0, 5 mM DTT, 200 mM NaCl) was mixed with reservoir (100 mM HEPES, pH 7.2, 28-30% (v/v) PEGMME2000, 200 mM $(\text{NH}_4)_2\text{SO}_4$, 20 mM β -ME) at a equal volume ratio of protein solution to reservoir and allowed to stand at room temperature. Prior to X-ray data collection at -169°C, a single crystal was equilibrated for 48 h in 100 mM HEPES pH 7.0, 200 mM $(\text{NH}_4)_2\text{SO}_4$, 28% PEGMME2000, 5% glycerol, 2% DMSO, and 1 mM SB203580.

X-ray data were collected on an Raxis IIC image plate and processed and scaled using DENZO and SCALEPACK [Z. Otwinowski et al., Meth. Enzymol., 276, pp. 307-326 (1996)]. The crystals had space group symmetry P21, with unit cell dimensions $a=48.6\text{\AA}$, $b=69.7\text{\AA}$, $c=60.3\text{\AA}$ and $b=109.25$. R-merge for the data was 3.2%, with $I/\text{sig}(I)=8.9$ at 1.95\AA resolution. The X-ray data comprised 26,737 unique reflections with $|F| > \sigma(F)$ derived from 69,783 intensity measurements. The data were 96.7% complete overall and 83.2% complete in the 2.01-1.95 Å resolution shell.

X-ray coordinates of unphosphorylated ERK2 were used to construct a model for the refinement of the inhibited ERK2(5X) complex. All thermal factors were set to 20.0\AA^2 . The R-factor after the rigid body and positional refinement was 30% for 10-2.4 Å data. The resolution of the maps and model was gradually increased to 2.0\AA resolution by cycles of model building, positional refinement, and thermal factor refinement,

interspersed with torsional dynamics runs. XPLOR was used for model refinement [A. T. Brunger, XPLOR: A system for X-ray crystallography & NMR, Ed., Yale University Press, New Haven, CT (Version 3.1)(1992)]. Our current
5 ERK2(5X) model in complex with SB203580 contains 334 protein residues, 283 water molecules, one sulfate molecule, and one inhibitor molecule, and has an R-factor of 21.3% (R-free = 28.6%) versus all data with $|F| > \sigma(F)$ between 6-2.0 Å resolution (23,621 reflections).

10 PROCHECK and XPLOR was used to analyze the model stereochemistry. Ninety percent of the ERK2 residues were located in the most favored region of the phi-psi plot, and 11% in the additional allowed regions. Deviations from ideal bond lengths and angles were 0.009Å
15 and 1.5° respectively, and other indications of stereochemistry were average or better than average for a structure determined at 2.0Å resolution. No electron density was observed for ERK2(5X) amino acids 1-13, 31-33, and 328-335, so these residues were not included in
20 the model.

The crystal structure revealed the interactions that lead to potent binding of the pyridinyl-imidazole compound, SB203580, with residues in the ATP site of ERK2(5X). The para-fluorophenyl ring of SB203580 was
25 shielded from solvent and was within favorable van der Waals distance ($<4.5\text{\AA}$) of the carbon atoms of eight ERK2 side chains; V37, A50, K52, I82, I84, L101, and T105. Comparing this structure with that of wild-type ERK2/ATP, showed that the larger glutamine side chain at position
30 105 in the wild-type protein would prohibit binding of SB203580 by blocking access to the pocket filled by the para-fluorophenyl ring.

Additional contacts were made between the pyridine ring and V39, A52, I84, L106, M108, and L156, while the 4-substituted phenyl ring of SB203580 contacted only L156 and C166. The interactions of the methane-sulfonyl group were more extensive, and this group was nearby to D167, N154, S153, and K151. The imidazole ring contacted V39, K54, L156 and C166, and appeared to assist in binding by positioning the three substituents.

Despite the high binding affinity, only one hydrogen bond was made between SB203580 and ERK2(5X).

EXAMPLE 6

Identification of the Amino Acid of Other MAP Kinases to Alter for Binding to Pyridinyl-Imidazole Compounds

The amino acid sequence of many other MAP kinases have been published. We have analyzed these sequences by protein alignment means and have determined the amino acid residue that aligns with threonine 106 of p38. If this amino acid is significantly different in character to threonine, then, by changing that amino acid to one with a small side chain (e.g., alanine or threonine), a mutant kinase can be created which can theoretically bind to a pyridinyl-imidazole inhibitor of p38. That complex can then be subjected to molecular modeling means which would allow for the design of an inhibitor of the corresponding native MAP kinase according to the methods of this invention.

This analysis is shown in the table below:

TABLE 3. Other MAP kinases for inhibitor design.

MAP Kinase	SEQ ID NO	Key Amino Acid
ERK6	24	methionine 109
ERK1	25	glutamine 122
p38- γ	26	methionine 107
p38- δ	27	methionine 107
JNK3- α 2	28	methionine 146
JNK2- α 1	29	methionine 108
JNK2- β 1	30	methionine 108
JNK2- β 2	31	methionine 108
JNK2	32	methionine 108
JNK1	33	methionine 108
JNK1- α 2	34	methionine 108
JNK1- β 1	35	methionine 108
JNK1- β 2	36	methionine 108
p38- β	37	threonine 106

While we have hereinbefore presented a number of embodiments of this invention, it is apparent
5 that our basic construction can be altered to provide other embodiments of this invention. Therefore, it will be appreciated that the scope of this invention is to be defined by the claims appended hereto rather than the specific embodiments which have been presented
10 hereinbefore by way of example.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Su, Michael Shin-San
Fox, Ted
5 Wilson, Keith Phillip
Germann, Ursula A.

(ii) TITLE OF INVENTION: Methods For Designing Inhibitors of
Serine/Thereonine Kinases and Tyrosine Kinase

10 (iii) NUMBER OF SEQUENCES: 37

(iv) CORRESPONDENCE ADDRESS:
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15 (C) CITY: New York
(D) STATE: NY
(E) COUNTRY: US
(F) ZIP: 10020

(v) COMPUTER READABLE FORM:
20 (A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
25 (A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
30 (A) NAME: Haley, James F.
(B) REGISTRATION NUMBER: 27,794
(C) REFERENCE/DOCKET NUMBER: VPI 97-104

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (212) 596-9000
(B) TELEFAX: (212) 596-9090

35 (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 360 amino acids
(B) TYPE: amino acid
40 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Met Ser Gln Glu Arg Pro Thr Phe Tyr Arg Gln Glu Leu Asn Lys Thr
1 5 10 15

	Ile Trp Glu Val Pro Glu Arg Tyr Gln Asn Leu Ser Pro Val Gly Ser	20	25	30
	Gly Ala Tyr Gly Ser Val Cys Ala Ala Phe Asp Thr Lys Thr Gly Leu	35	40	45
5	Arg Val Ala Val Lys Lys Leu Ser Arg Pro Phe Gln Ser Ile Ile His	50	55	60
	Ala Lys Arg Thr Tyr Arg Glu Leu Arg Leu Leu Lys His Met Lys His	65	70	75
10	Glu Asn Val Ile Gly Leu Leu Asp Val Phe Thr Pro Ala Arg Ser Leu	85	90	95
	Glu Glu Phe Asn Asp Val Tyr Leu Val Thr His Leu Met Gly Ala Asp	100	105	110
	Leu Asn Asn Ile Val Lys Cys Gln Lys Leu Thr Asp Asp His Val Gln	115	120	125
15	Phe Leu Ile Tyr Gln Ile Leu Arg Gly Leu Lys Tyr Ile His Ser Ala	130	135	140
	Asp Ile Ile His Arg Asp Leu Lys Pro Ser Asn Leu Ala Val Asn Glu	145	150	155
20	Asp Cys Glu Leu Lys Ile Leu Asp Phe Gly Leu Ala Arg His Thr Asp	165	170	175
	Asp Glu Met Thr Gly Tyr Val Ala Thr Arg Trp Tyr Arg Ala Pro Glu	180	185	190
	Ile Met Leu Asn Trp Met His Tyr Asn Gln Thr Val Asp Ile Trp Ser	195	200	205
25	Val Gly Cys Ile Met Ala Glu Leu Leu Thr Gly Arg Thr Leu Phe Pro	210	215	220
	Gly Thr Asp His Ile Asp Gln Leu Lys Leu Ile Leu Arg Leu Val Gly	225	230	235
30	Thr Pro Gly Ala Glu Leu Leu Lys Lys Ile Ser Ser Glu Ser Ala Arg	245	250	255
	Asn Tyr Ile Gln Ser Leu Thr Gln Met Pro Lys Met Asn Phe Ala Asn	260	265	270
	Val Phe Ile Gly Ala Asn Pro Leu Ala Val Asp Leu Leu Glu Lys Met	275	280	285
35	Leu Val Leu Asp Ser Asp Lys Arg Ile Thr Ala Ala Gln Ala Leu Ala	290	295	300
	His Ala Tyr Phe Ala Gln Tyr His Asp Pro Asp Asp Glu Pro Val Ala	305	310	315
				320

CCCTCC "CCTCC" C

Asp Pro Tyr Asp Gln Ser Phe Glu Ser Arg Asp Leu Leu Ile Asp Glu
325 330 335

Trp Lys Ser Leu Thr Tyr Asp Glu Val Ile Ser Phe Val Pro Pro Pro
340 345 350

5 Leu Asp Gln Glu Glu Met Glu Ser
355 360

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
10 (A) LENGTH: 360 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:
15 (A) NAME/KEY: Region
(B) LOCATION: 103..104
(D) OTHER INFORMATION: /note= "amino acid 103 is
isoleucine or leucine"

(ix) FEATURE:
20 (A) NAME/KEY: Region
(B) LOCATION: 105..106
(D) OTHER INFORMATION: /product= "OTHER"
/note= "amino acid 105 is glutamine, threonine or alanine"

(ix) FEATURE:
25 (A) NAME/KEY: Region
(B) LOCATION: 106..107
(D) OTHER INFORMATION: /product= "OTHER"
/note= "amino acid 106 is aspartic acid or histidine"

(ix) FEATURE:
30 (A) NAME/KEY: Region
(B) LOCATION: 109..110
(D) OTHER INFORMATION: /product= "OTHER"
/note= "amino acid 109 is glutamic acid or glycine"

(ix) FEATURE:
35 (A) NAME/KEY: Region
(B) LOCATION: 110..111
(D) OTHER INFORMATION: /product= "OTHER"
/note= "amino acid 110 is threonine or alanine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

40 Met Ala Ala Ala Ala Ala Ala Gly Ala Gly Pro Glu Met Val Arg Gly
1 5 10 15

Gln Val Phe Asp Val Gly Pro Arg Tyr Thr Asn Leu Ser Tyr Ile Gly
20 25 30

	Glu	Gly	Ala	Tyr	Gly	Met	Val	Cys	Ser	Ala	Tyr	Asp	Asn	Val	Asn	Lys	
			35					40					45				
	Val	Arg	Val	Ala	Ile	Lys	Lys	Ile	Ser	Pro	Phe	Glu	His	Gln	Thr	Tyr	
		50					55					60					
5	Cys	Gln	Arg	Thr	Leu	Arg	Glu	Ile	Lys	Ile	Leu	Leu	Arg	Phe	Arg	His	
		65				70					75					80	
	Glu	Asn	Ile	Ile	Gly	Ile	Asn	Asp	Ile	Ile	Arg	Ala	Pro	Thr	Ile	Glu	
					85					90					95		
10	Gln	Met	Lys	Asp	Val	Tyr	Xaa	Val	Xaa	Xaa	Leu	Met	Xaa	Xaa	Asp	Leu	
				100					105						110		
	Tyr	Lys	Leu	Leu	Lys	Thr	Gln	His	Leu	Ser	Asn	Asp	His	Ile	Cys	Tyr	
			115					120					125				
	Phe	Leu	Tyr	Gln	Ile	Leu	Arg	Gly	Leu	Lys	Tyr	Ile	His	Ser	Ala	Asn	
		130					135					140					
15	Val	Leu	His	Arg	Asp	Leu	Lys	Pro	Ser	Asn	Leu	Leu	Leu	Asn	Thr	Thr	
		145				150					155					160	
	Cys	Asp	Leu	Lys	Ile	Cys	Asp	Phe	Gly	Leu	Ala	Arg	Val	Ala	Asp	Pro	
				165						170					175		
20	Asp	His	Asp	His	Thr	Gly	Phe	Leu	Thr	Glu	Tyr	Val	Ala	Thr	Arg	Trp	
				180					185					190			
	Tyr	Arg	Ala	Pro	Glu	Ile	Met	Leu	Asn	Ser	Lys	Gly	Tyr	Thr	Lys	Ser	
			195				200						205				
	Ile	Asp	Ile	Trp	Ser	Val	Gly	Cys	Ile	Leu	Ala	Glu	Met	Leu	Ser	Asn	
		210					215					220					
25	Arg	Pro	Ile	Phe	Pro	Gly	Lys	His	Tyr	Leu	Asp	Gln	Leu	Asn	His	Ile	
		225				230					235					240	
	Leu	Gly	Ile	Leu	Gly	Ser	Pro	Ser	Gln	Glu	Asp	Leu	Asn	Cys	Ile	Ile	
				245						250					255		
30	Asn	Leu	Lys	Ala	Arg	Asn	Tyr	Leu	Leu	Ser	Leu	Pro	His	Lys	Asn	Lys	
			260						265					270			
	Val	Pro	Trp	Asn	Arg	Leu	Phe	Pro	Asn	Ala	Asp	Ser	Lys	Ala	Leu	Asp	
			275					280					285				
	Leu	Leu	Asp	Lys	Met	Leu	Thr	Phe	Asn	Pro	His	Lys	Arg	Ile	Glu	Val	
		290					295					300					
35	Glu	Gln	Ala	Leu	Ala	His	Pro	Tyr	Leu	Glu	Gln	Tyr	Tyr	Asp	Pro	Ser	
		305				310					315					320	
	Asp	Glu	Pro	Ile	Ala	Glu	Ala	Pro	Phe	Lys	Phe	Asp	Met	Glu	Leu	Asp	
				325						330					335		

Asp Leu Pro Lys Glu Lys Leu Lys Glu Leu Ile Phe Glu Glu Thr Ala
340 345 350

Arg Phe Gln Pro Gly Tyr Arg Ser
355 360

5 (2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- 10 (A) LENGTH: 422 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(ix) FEATURE:

- 15 (A) NAME/KEY: Region
(B) LOCATION: 146..147
(D) OTHER INFORMATION: /product= "OTHER"
/note= "amino acid 146 is methionine, threonine or alanine"

(ix) FEATURE:

- 20 (A) NAME/KEY: Region
(B) LOCATION: 150..151
(D) OTHER INFORMATION: /product= "OTHER"
/note= "amino acid 150 is aspartic acid or glycine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

25 Met Ser Leu His Phe Leu Tyr Tyr Cys Ser Glu Pro Thr Leu Asp Val
1 5 10 15
Lys Ile Ala Phe Cys Gln Gly Phe Asp Lys Gln Val Asp Val Ser Tyr
20 25 30
Ile Ala Lys His Tyr Asn Met Ser Lys Ser Lys Val Asp Asn Gln Phe
35 40 45
30 Tyr Ser Val Glu Val Gly Asp Ser Thr Phe Thr Val Leu Lys Arg Tyr
50 55 60
Gln Asn Leu Lys Pro Ile Gly Ser Gly Ala Gln Gly Ile Val Cys Ala
65 70 75 80
35 Ala Tyr Asp Ala Val Leu Asp Arg Asn Val Ala Ile Lys Lys Leu Ser
85 90 95
Arg Pro Phe Gln Asn Gln Thr His Ala Lys Arg Ala Tyr Arg Glu Leu
100 105 110
Val Leu Met Lys Cys Val Asn His Lys Asn Ile Ile Ser Leu Leu Asn
115 120 125
40 Val Phe Thr Pro Gln Lys Thr Leu Glu Glu Phe Gln Asp Val Tyr Leu
130 135 140

	Val Xaa Glu Leu Met Xaa Ala Asn Leu Cys Gln Val Ile Gln Met Glu	145	150	155	160
	Leu Asp His Glu Arg Met Ser Tyr Leu Leu Tyr Gln Met Leu Cys Gly	165	170	175	
5	Ile Lys His Leu His Ser Ala Gly Ile Ile His Arg Asp Leu Lys Pro	180	185	190	
	Ser Asn Ile Val Val Lys Ser Asp Cys Thr Leu Lys Ile Leu Asp Phe	195	200	205	
10	Gly Leu Ala Arg Thr Ala Gly Thr Ser Phe Met Met Thr Pro Tyr Val	210	215	220	
	Val Thr Arg Tyr Tyr Arg Ala Pro Glu Val Ile Leu Gly Met Gly Tyr	225	230	235	240
	Lys Glu Asn Val Asp Ile Trp Ser Val Gly Cys Ile Met Gly Glu Met	245	250	255	
15	Val Arg His Lys Ile Leu Phe Pro Gly Arg Asp Tyr Ile Asp Gln Trp	260	265	270	
	Asn Lys Val Ile Glu Gln Leu Gly Thr Pro Cys Pro Glu Phe Met Lys	275	280	285	
20	Lys Leu Gln Pro Thr Val Arg Asn Tyr Val Glu Asn Arg Pro Lys Tyr	290	295	300	
	Ala Gly Leu Thr Phe Pro Lys Leu Phe Pro Asp Ser Leu Phe Pro Ala	305	310	315	320
	Asp Ser Glu His Asn Lys Leu Lys Ala Ser Gln Ala Arg Asp Leu Leu	325	330	335	
25	Ser Lys Met Leu Val Ile Asp Pro Ala Lys Arg Ile Ser Val Asp Asp	340	345	350	
	Ala Leu Gln His Pro Tyr Ile Asn Val Trp Tyr Asp Pro Ala Glu Val	355	360	365	
30	Glu Ala Pro Pro Pro Gln Ile Tyr Asp Lys Gln Leu Asp Glu Arg Glu	370	375	380	
	His Thr Ile Glu Glu Trp Lys Glu Leu Ile Tyr Lys Glu Val Met Asn	385	390	395	400
	Ser Glu Glu Lys Thr Lys Asn Gly Val Val Lys Gly Gln Pro Ser Pro	405	410	415	
35	Ser Ala Gln Val Gln Gln	420			

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

```

      (A) LENGTH: 30 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

5      (ii) MOLECULE TYPE: other nucleic acid
      (A) DESCRIPTION: /desc = "oligonucleotide"

      (iii) HYPOTHETICAL: NO

      (iv) ANTI-SENSE: NO

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
10  GAACGGCGGG CAGCCAACAT GGCGGCGGCG                                     30

      (2) INFORMATION FOR SEQ ID NO:5:

      (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 36 base pairs
      (B) TYPE: nucleic acid
15  (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: other nucleic acid
      (A) DESCRIPTION: /desc = "oligonucleotide"

      (iii) HYPOTHETICAL: NO

20  (iv) ANTI-SENSE: NO

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

      GGGCTCGAGC CTGACAAATT TAAGATCTGT ATCCTG                                     36

      (2) INFORMATION FOR SEQ ID NO:6:

      (i) SEQUENCE CHARACTERISTICS:
25  (A) LENGTH: 28 base pairs
      (B) TYPE: nucleic acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

      (ii) MOLECULE TYPE: other nucleic acid
30  (A) DESCRIPTION: /desc = "oligonucleotide"

      (iii) HYPOTHETICAL: NO

      (iv) ANTI-SENSE: NO

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

      TTAACATATG GCGGCGGCGG CGGCGGCG                                     28

35  (2) INFORMATION FOR SEQ ID NO:7:

      (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 25 base pairs

```

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
5 (A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCCACAGGAT CCGATCTGTA TCCTG 25

10 (2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
15 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GATGGTCCGC GGGCAGGTGT TCG 23

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
25 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

30 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GTGTCTTCAA AAGCTTGTA AGATCTGTTT CC 32

(2) INFORMATION FOR SEQ ID NO:10:

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

5 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CAAAAGCTTG TAAAGATCTG TTTCCATGAG GTCCGTTACT AT

42

(2) INFORMATION FOR SEQ ID NO:11:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

20 CAAAAGCTTG TAAAGATCTG TTTCCATGAG GTCCGTTACT AT

42

(2) INFORMATION FOR SEQ ID NO:12:

25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: NO

30 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

CAAAAGCTTG TAAAGATCTG CTCCCATGAG GTGCGTTACT AGATATAC

48

(2) INFORMATION FOR SEQ ID NO:13:

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: NO

5 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GATCTTTACA AGCTTTTGAA GACACAAC 28

(2) INFORMATION FOR SEQ ID NO:14:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CTTGGTGTAG CCCTTGGAAT TCAACATA 28

20 (2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
25 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: C-terminal

30 (ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION: 1..6
(D) OTHER INFORMATION: /note= "C-terminal tag"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

35 Glu Tyr Met Pro Met Glu
1 5

(2) INFORMATION FOR SEQ ID NO:16.

- 5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"
- (iii) HYPOTHETICAL: NO

10 (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CATGGCACAC CATCACCATC ACCATCCCAA GAAGAAGCCG ACGCCCATCC AG 52

(2) INFORMATION FOR SEQ ID NO:17:

- 15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
20 (A) DESCRIPTION: /desc = "oligonucleotide"
- (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CTGGATGGGC GTCGGCTTCT TCTTGGGATG GTGATGGTGA TGGTGTGC 48

25 (2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
30 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"
- (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GCTCTAGAGC TCCATGGGCA GCAAAAGCAA AGTTGACAA 39

CCCTGGATGGGC

(2) INFORMATION FOR SEQ ID NO:19:

- 5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"
- (iii) HYPOTHETICAL: NO
- 10 (iv) ANTI-SENSE: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TAGCGGATCC TCATTCTGAA TTCATTACTT CCTTGTA

37

(2) INFORMATION FOR SEQ ID NO:20:

- 15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
20 (A) DESCRIPTION: /desc = "oligonucleotide"
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

CCAAGATGTT TACTTAGTAG CGGAAGTAT GGATGCCAA

39

25 (2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
30 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "oligonucleotide"
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- 35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CAAGATGTTT ACTTAGTAAC GGGACTGATG GATGCCAAC

39

(2) INFORMATION FOR SEQ ID NO:22:

- 5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 36 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: other nucleic acid
 (A) DESCRIPTION: /desc = "oligonucleotide"
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GTAATGGAAC TGATGGGTGC CAACTTATGT CAAGTG

36

(2) INFORMATION FOR SEQ ID NO:23:

- 15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- 20 (iv) ANTI-SENSE: NO
- (ix) FEATURE:
 (A) NAME/KEY: Peptide
 (B) LOCATION: 1..21
 (D) OTHER INFORMATION: /note= "EGF receptor peptide"

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Lys Arg Glu Leu Val Glu Pro Leu Thr Pro Ser Gly Glu Ala Pro Asn
 1 5 10 15

Gln Ala Leu Leu Arg
 20

30 (2) INFORMATION FOR SEQ ID NO:24:

- 35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 367 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

	Met	Ser	Ser	Pro	Pro	Pro	Thr	Arg	Ser	Gly	Phe	Tyr	Arg	Gln	Glu	Val	
	1				5					10					15		
	Thr	Lys	Thr	Ala	Trp	Glu	Val	Arg	Ala	Val	Tyr	Arg	Asp	Leu	Gln	Pro	
				20					25					30			
5	Val	Gly	Ser	Gly	Ala	Tyr	Gly	Ala	Val	Cys	Ser	Ala	Val	Asp	Gly	Arg	
			35					40					45				
	Thr	Gly	Ala	Lys	Val	Ala	Ile	Lys	Lys	Leu	Tyr	Arg	Pro	Phe	Gln	Ser	
		50					55					60					
10	Glu	Leu	Phe	Ala	Lys	Leu	Ala	Tyr	Arg	Glu	Leu	Arg	Leu	Leu	Lys	His	
	65					70					75				80		
	Met	Arg	His	Glu	Asn	Val	Ile	Gly	Leu	Leu	Asp	Val	Phe	Thr	Pro	Asp	
					85					90					95		
	Glu	Thr	Leu	Asp	Asp	Phe	Thr	Asp	Phe	Tyr	Leu	Val	Met	Pro	Phe	Met	
				100					105					110			
15	Gly	Thr	Asp	Leu	Gly	Lys	Leu	Met	Lys	His	Glu	Lys	Leu	Gly	Glu	Asp	
			115					120					125				
	Arg	Ile	Gln	Phe	Leu	Val	Tyr	Gln	Met	Met	Lys	Gly	Leu	Arg	Tyr	Ile	
		130					135					140					
20	His	Ala	Ala	Gly	Ile	Ile	His	Arg	Asp	Leu	Lys	Pro	Gly	Asn	Leu	Ala	
	145					150					155				160		
	Val	Asn	Glu	Asp	Cys	Glu	Leu	Lys	Ile	Leu	Asp	Phe	Gly	Leu	Ala	Arg	
					165					170					175		
	Gln	Ala	Asp	Ser	Glu	Met	Thr	Gly	Tyr	Val	Val	Thr	Arg	Trp	Tyr	Arg	
				180					185					190			
25	Ala	Pro	Glu	Val	Ile	Leu	Asn	Trp	Ile	Ala	Tyr	Thr	Gln	Thr	Val	Asp	
			195					200					205				
	Ile	Trp	Ser	Val	Gly	Cys	Ile	Met	Ala	Glu	Met	Ile	Thr	Gly	Lys	Thr	
		210					215					220					
30	Leu	Phe	Lys	Gly	Ser	Asp	His	Leu	Asp	Gln	Leu	Lys	Glu	Ile	Met	Lys	
	225					230					235				240		
	Val	Thr	Gly	Thr	Pro	Pro	Ala	Glu	Phe	Val	Gln	Arg	Leu	Gln	Ser	Asp	
					245					250					255		
	Glu	Ala	Lys	Asn	Tyr	Met	Lys	Gly	Leu	Pro	Glu	Leu	Glu	Lys	Lys	Asp	
				260					265				270				
35	Phe	Ala	Ser	Ile	Leu	Thr	Asn	Ala	Ser	Pro	Leu	Ala	Val	Asn	Leu	Leu	
			275					280					285				
	Glu	Lys	Met	Leu	Val	Leu	Asp	Ala	Asp	Ile	Arg	Leu	Thr	Ala	Gly	Glu	
		290					295					300					

Phe	Leu	Ser	His	Pro	Tyr	Phe	Glu	Ser	Leu	His	Asp	Thr	Glu	Asp	Glu	
305					310					315					320	
Pro	Gln	Val	Gln	Lys	Tyr	Asp	Asp	Ser	Phe	Asp	Tyr	Phe	Asp	Arg	Thr	
				325					330					335		
5	Leu	Asp	Glu	Trp	Lys	Arg	Val	Thr	Tyr	Lys	Glu	Val	Leu	Ser	Phe	Lys
				340					345					350		
Pro	Pro	Arg	Gln	Leu	Gly	Ala	Arg	Val	Ser	Lys	Glu	Thr	Pro	Leu		
				355			360					365				

(2) INFORMATION FOR SEQ ID NO:25:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 379 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Met	Ala	Ala	Ala	Ala	Ala	Gln	Gly	Gly	Gly	Gly	Gly	Glu	Pro	Arg	Arg	
1					5					10				15		
Thr	Glu	Gly	Val	Gly	Pro	Gly	Val	Pro	Gly	Glu	Val	Glu	Met	Val	Lys	
20				20				25					30			
Gly	Gln	Pro	Phe	Asp	Val	Gly	Pro	Arg	Tyr	Thr	Gln	Leu	Gln	Tyr	Ile	
				35			40					45				
Gly	Glu	Gly	Ala	Tyr	Gly	Met	Val	Ser	Ser	Ala	Tyr	Asp	His	Val	Arg	
				50			55				60					
25	Lys	Thr	Arg	Val	Ala	Ile	Lys	Lys	Ile	Ser	Pro	Phe	Glu	His	Gln	Thr
	65					70				75					80	
Tyr	Cys	Gln	Arg	Thr	Leu	Arg	Glu	Ile	Gln	Ile	Leu	Leu	Arg	Phe	Arg	
				85				90						95		
His	Glu	Asn	Val	Ile	Gly	Ile	Arg	Asp	Ile	Leu	Arg	Ala	Ser	Thr	Leu	
30				100				105					110			
Glu	Ala	Met	Arg	Asp	Val	Tyr	Ile	Val	Gln	Asp	Leu	Met	Glu	Thr	Asp	
				115			120					125				
Leu	Tyr	Lys	Leu	Leu	Lys	Ser	Gln	Gln	Leu	Ser	Asn	Asp	His	Ile	Cys	
				130			135				140					
35	Tyr	Phe	Leu	Tyr	Gln	Ile	Leu	Arg	Gly	Leu	Lys	Tyr	Ile	His	Ser	Ala
	145					150					155					160
Asn	Val	Leu	His	Arg	Asp	Leu	Lys	Pro	Ser	Asn	Leu	Leu	Ser	Asn	Thr	
				165					170						175	

	Thr	Cys	Asp	Leu	Lys	Ile	Cys	Asp	Phe	Gly	Leu	Ala	Arg	Ile	Ala	Asp	160	185	190
	Pro	Glu	His	Asp	His	Thr	Gly	Phe	Leu	Thr	Glu	Tyr	Val	Ala	Thr	Arg	195	200	205
5	Trp	Tyr	Arg	Ala	Pro	Glu	Ile	Met	Leu	Asn	Ser	Lys	Gly	Tyr	Thr	Lys	210	215	220
	Ser	Ile	Asp	Ile	Trp	Ser	Val	Gly	Cys	Ile	Leu	Ala	Glu	Met	Leu	Ser	225	230	235
10	Asn	Arg	Pro	Ile	Phe	Pro	Gly	Lys	His	Tyr	Leu	Asp	Gln	Leu	Asn	His	245	250	255
	Ile	Leu	Gly	Ile	Leu	Gly	Ser	Pro	Ser	Gln	Glu	Asp	Leu	Asn	Cys	Ile	260	265	270
	Ile	Asn	Met	Lys	Ala	Arg	Asn	Tyr	Leu	Gln	Ser	Leu	Pro	Ser	Lys	Thr	275	280	285
15	Lys	Val	Ala	Trp	Ala	Lys	Leu	Phe	Pro	Lys	Ser	Asp	Ser	Lys	Ala	Leu	290	295	300
	Asp	Leu	Leu	Asp	Arg	Met	Leu	Thr	Phe	Asn	Pro	Asn	Lys	Arg	Ile	Thr	305	310	315
20	Val	Glu	Glu	Ala	Leu	Ala	His	Pro	Tyr	Leu	Glu	Gln	Tyr	Tyr	Asp	Pro	325	330	335
	Thr	Asp	Glu	Pro	Val	Ala	Glu	Glu	Pro	Phe	Thr	Phe	Ala	Met	Glu	Leu	340	345	350
	Asp	Asp	Leu	Pro	Lys	Glu	Arg	Leu	Lys	Glu	Leu	Ile	Phe	Gln	Glu	Thr	355	360	365
25	Ala	Arg	Phe	Gln	Pro	Gly	Val	Leu	Glu	Ala	Pro						370	375	

(2) INFORMATION FOR SEQ ID NO:26:

	(i)	SEQUENCE CHARACTERISTICS:																		
30	(A)	LENGTH: 365 amino acids																		
	(B)	TYPE: amino acid																		
	(C)	STRANDEDNESS: single																		
	(D)	TOPOLOGY: linear																		
	(ii)	MOLECULE TYPE: protein																		
	(xi)	SEQUENCE DESCRIPTION: SEQ ID NO:26:																		
35	Met	Ser	Leu	Ile	Arg	Lys	Lys	Gly	Phe	Tyr	Lys	Gln	Glu	Leu	Asn	Lys	1	5	10	15
	Thr	Ala	Trp	Glu	Leu	Pro	Lys	Thr	Tyr	Val	Ser	Pro	Thr	His	Val	Gly	20	25	30	

	Ser	Gly	Ala	Tyr	Gly	Ser	Trp	Cys	Ser	Ala	Ile	Asp	Lys	Arg	Ser	Gly	
	35						40					45					
	Glu	Lys	Val	Ala	Ile	Lys	Lys	Leu	Ser	Arg	Pro	Phe	Gln	Ser	Glu	Ile	
	50					55						60					
5	Phe	Ala	Lys	Arg	Ala	Tyr	Arg	Glu	Leu	Leu	Leu	Leu	Lys	His	Met	Gln	
	65					70					75					80	
	His	Glu	Asn	Val	Ile	Gly	Leu	Leu	Asp	Val	Phe	Thr	Pro	Ala	Ser	Ser	
				85						90					95		
10	Leu	Arg	Asn	Phe	Tyr	Asp	Phe	Tyr	Leu	Val	Met	Pro	Phe	Met	Gln	Thr	
			100						105					110			
	Asp	Leu	Gln	Lys	Ile	Met	Gly	Met	Glu	Phe	Ser	Glu	Glu	Lys	Ile	Gln	
			115					120					125				
	Tyr	Leu	Val	Tyr	Gln	Met	Leu	Lys	Gly	Leu	Lys	Tyr	Ile	His	Ser	Ala	
	130						135					140					
15	Gly	Val	Val	His	Arg	Asp	Leu	Lys	Pro	Gly	Asn	Leu	Ala	Val	Asn	Glu	
	145					150					155					160	
	Asp	Cys	Glu	Leu	Lys	Ile	Leu	Asp	Phe	Gly	Leu	Ala	Arg	His	Ala	Asp	
					165					170					175		
20	Ala	Glu	Met	Thr	Gly	Tyr	Val	Val	Thr	Arg	Trp	Tyr	Arg	Ala	Pro	Glu	
				180					185					190			
	Val	Ile	Leu	Ser	Trp	Met	His	Tyr	Asn	Gln	Thr	Val	Asp	Ile	Trp	Ser	
			195					200					205				
	Val	Gly	Cys	Ile	Met	Ala	Glu	Met	Leu	Thr	Gly	Lys	Thr	Leu	Phe	Lys	
		210					215					220					
25	Gly	Lys	Asp	Tyr	Leu	Asp	Gln	Leu	Thr	Gln	Ile	Leu	Lys	Val	Thr	Gly	
	225					230					235					240	
	Val	Pro	Gly	Thr	Glu	Phe	Val	Gln	Lys	Leu	Asn	Asp	Lys	Ala	Ala	Lys	
					245					250					255		
30	Ser	Tyr	Ile	Gln	Ser	Leu	Pro	Gln	Thr	Pro	Arg	Lys	Asp	Phe	Thr	Gln	
				260					265					270			
	Leu	Phe	Pro	Arg	Ala	Ser	Pro	Gln	Ala	Ala	Asp	Leu	Leu	Glu	Lys	Met	
			275					280					285				
	Leu	Glu	Leu	Asp	Val	Asp	Lys	Arg	Leu	Thr	Ala	Ala	Gln	Ala	Leu	Thr	
		290					295					300					
35	His	Pro	Phe	Phe	Glu	Pro	Phe	Arg	Asp	Pro	Glu	Glu	Glu	Thr	Glu	Ala	
	305					310					315					320	
	Gln	Gln	Pro	Phe	Asp	Asp	Ser	Leu	Glu	His	Glu	Lys	Leu	Thr	Val	Asp	
					325					330					335		

Glu Trp Lys Gln His Ile Tyr Lys Glu Ile Val Asn Phe Ser Pro Ile
340 345 350

Ala Arg Lys Asp Ser Arg Arg Arg Ser Gly Met Lys Leu
355 360 365

5 (2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 365 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Met Ser Leu Ile Arg Lys Lys Gly Phe Tyr Lys Gln Asp Val Asn Lys
1 5 10 15

Thr Ala Trp Glu Leu Pro Lys Thr Tyr Val Ser Pro Thr His Val Gly
20 25 30

Ser Gly Ala Tyr Gly Ser Val Cys Ser Ala Ile Asp Lys Arg Ser Gly
35 40 45

Glu Lys Val Ala Ile Lys Lys Leu Ser Arg Pro Phe Gln Ser Glu Ile
50 55 60

Phe Ala Lys Arg Ala Tyr Arg Glu Leu Leu Leu Lys His Met Gln
65 70 75 80

His Glu Asn Val Ile Gly Leu Leu Asp Val Phe Thr Pro Ala Ser Ser
85 90 95

Leu Arg Asn Phe Tyr Asp Phe Tyr Leu Val Met Pro Phe Met Gln Thr
100 105 110

Asp Leu Gln Lys Ile Met Gly Met Glu Phe Ser Glu Glu Lys Ile Gln
115 120 125

Tyr Leu Val Tyr Gln Met Leu Lys Gly Leu Lys Tyr Ile His Ser Ala
130 135 140

Gly Val Val His Arg Asp Leu Lys Pro Gly Asn Leu Ala Val Asn Glu
145 150 155 160

Asp Cys Glu Leu Lys Ile Leu Asp Phe Gly Leu Ala Arg His Ala Asp
165 170 175

Ala Glu Met Thr Gly Tyr Val Val Thr Arg Trp Tyr Arg Ala Pro Glu
180 185 190

Val Ile Leu Ser Trp Met His Tyr Asn Gln Thr Val Asp Ile Trp Ser
195 200 205

	Val	Gly	Cys	Ile	Met	Ala	Glu	Met	Leu	Thr	Gly	Lys	Thr	Leu	Phe	Lys	
	210						215					220					
	Gly	Lys	Asp	Tyr	Leu	Asp	Gln	Leu	Thr	Gln	Ile	Leu	Lys	Val	Thr	Gly	
	225					230					235					240	
5	Val	Pro	Gly	Thr	Glu	Phe	Val	Gln	Lys	Leu	Asn	Asp	Lys	Ala	Ala	Lys	
					245					250					255		
	Ser	Tyr	Ile	Gln	Ser	Leu	Pro	Gln	Thr	Pro	Arg	Lys	Asp	Phe	Thr	Gln	
				260					265					270			
10	Leu	Phe	Pro	Arg	Ala	Ser	Pro	Gln	Ala	Ala	Asp	Leu	Leu	Glu	Lys	Met	
			275					280					285				
	Leu	Glu	Leu	Asp	Val	Asp	Lys	Arg	Leu	Thr	Ala	Ala	Gln	Ala	Leu	Thr	
			290				295					300					
	His	Pro	Phe	Phe	Glu	Pro	Phe	Arg	Asp	Pro	Glu	Glu	Glu	Thr	Glu	Ala	
	305					310					315					320	
15	Gln	Gln	Pro	Phe	Asp	Asp	Ser	Leu	Glu	His	Glu	Lys	Leu	Thr	Val	Asp	
					325					330					335		
	Glu	Trp	Lys	Gln	His	Ile	Tyr	Lys	Glu	Ile	Val	Asn	Phe	Ser	Pro	Ile	
				340					345				350				
20	Ala	Arg	Lys	Asp	Ser	Arg	Arg	Arg	Ser	Gly	Met	Lys	Leu				
			355				360						365				

(2) INFORMATION FOR SEQ ID NO:28:

	(i) SEQUENCE CHARACTERISTICS:																
	(A) LENGTH: 464 amino acids																
	(B) TYPE: amino acid																
25	(C) STRANDEDNESS: single																
	(D) TOPOLOGY: linear																
	(ii) MOLECULE TYPE: protein																
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:																
30	Met	Ser	Leu	His	Phe	Leu	Tyr	Tyr	Cys	Ser	Glu	Pro	Thr	Leu	Asp	Val	
	1				5					10					15		
	Lys	Ile	Ala	Phe	Cys	Gln	Gly	Phe	Asp	Lys	Gln	Val	Asp	Val	Ser	Tyr	
					20				25					30			
	Ile	Ala	Lys	His	Tyr	Asn	Met	Ser	Lys	Ser	Lys	Val	Asp	Asn	Gln	Phe	
			35					40					45				
35	Tyr	Ser	Val	Glu	Val	Gly	Asp	Ser	Thr	Phe	Thr	Val	Leu	Lys	Arg	Tyr	
		50				55						60					
	Gln	Asn	Leu	Lys	Pro	Ile	Gly	Ser	Gly	Ala	Gln	Gly	Ile	Val	Cys	Ala	
	65					70				75						80	

	Ala Tyr Asp Ala Val Leu Asp Arg Asn Val Ala Ile Lys Lys Leu Ser	85	90	95
	Arg Pro Phe Gln Asn Gln Thr His Ala Lys Arg Ala Tyr Arg Glu Leu	100	105	110
5	Val Leu Met Lys Cys Val Asn His Lys Asn Ile Ile Ser Leu Leu Asn	115	120	125
	Val Phe Thr Pro Gln Lys Thr Leu Glu Glu Phe Gln Asp Val Tyr Leu	130	135	140
10	Val Met Glu Leu Met Asp Ala Asn Leu Cys Gln Val Ile Gln Met Glu	145	150	155
	Leu Asp His Glu Arg Met Ser Tyr Leu Leu Tyr Gln Met Leu Cys Gly	165	170	175
	Ile Lys His Leu His Ser Ala Gly Ile Ile His Arg Asp Leu Lys Pro	180	185	190
15	Ser Asn Ile Val Val Lys Ser Asp Cys Thr Leu Lys Ile Leu Asp Phe	195	200	205
	Gly Leu Ala Arg Thr Ala Gly Thr Ser Phe Met Met Thr Pro Tyr Val	210	215	220
20	Val Thr Arg Tyr Tyr Arg Ala Pro Glu Val Ile Leu Gly Met Gly Tyr	225	230	235
	Lys Glu Asn Val Asp Ile Trp Ser Val Gly Cys Ile Met Gly Glu Met	245	250	255
	Val Arg His Lys Ile Leu Phe Pro Gly Arg Asp Tyr Ile Asp Gln Trp	260	265	270
25	Asn Lys Val Ile Glu Gln Leu Gly Thr Pro Cys Pro Glu Phe Met Lys	275	280	285
	Lys Leu Gln Pro Thr Val Arg Asn Tyr Val Glu Asn Arg Pro Lys Tyr	290	295	300
30	Ala Gly Leu Thr Phe Pro Lys Leu Phe Pro Asp Ser Leu Phe Pro Ala	305	310	315
	Asp Ser Glu His Asn Lys Leu Lys Ala Ser Gln Ala Arg Asp Leu Leu	325	330	335
	Ser Lys Met Leu Val Ile Asp Pro Ala Lys Arg Ile Ser Val Asp Asp	340	345	350
35	Ala Leu Gln His Pro Tyr Ile Asn Val Trp Tyr Asp Pro Ala Glu Val	355	360	365
	Glu Ala Pro Pro Pro Gln Ile Tyr Asp Lys Gln Leu Asp Glu Arg Glu	370	375	380

	His	Thr	Ile	Glu	Glu	Trp	Lys	Glu	Leu	Ile	Tyr	Lys	Glu	Val	Met	Asn
	385					390					395					400
	Ser	Glu	Glu	Lys	Thr	Lys	Asn	Gly	Val	Val	Lys	Gly	Gln	Pro	Ser	Pro
					405					410					415	
5	Ser	Gly	Ala	Ala	Val	Asn	Ser	Ser	Glu	Ser	Leu	Pro	Pro	Ser	Ser	Ser
					420				425					430		
	Val	Asn	Asp	Ile	Ser	Ser	Met	Ser	Thr	Asp	Gln	Thr	Leu	Ala	Ser	Asp
			435					440					445			
10	Thr	Asp	Ser	Ser	Leu	Glu	Ala	Ser	Ala	Gly	Pro	Leu	Gly	Cys	Cys	Arg
	450						455					460				

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 382 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

20	Met	Ser	Asp	Ser	Lys	Cys	Asp	Ser	Gln	Phe	Tyr	Ser	Val	Gln	Val	Ala
	1				5					10					15	
	Asp	Ser	Thr	Phe	Thr	Val	Leu	Lys	Arg	Tyr	Gln	Gln	Leu	Lys	Pro	Ile
				20					25					30		
	Gly	Ser	Gly	Ala	Gln	Gly	Ile	Val	Cys	Ala	Ala	Phe	Asp	Thr	Val	Leu
			35					40					45			
25	Gly	Ile	Ser	Val	Ala	Val	Lys	Lys	Leu	Ser	Arg	Pro	Phe	Gln	Asn	Gln
		50					55					60				
	Thr	His	Ala	Lys	Arg	Ala	Tyr	Arg	Glu	Leu	Val	Leu	Leu	Lys	Cys	Val
	65				70						75					80
30	Asn	His	Lys	Asn	Ile	Ile	Ser	Leu	Leu	Asn	Val	Phe	Thr	Pro	Gln	Lys
				85						90					95	
	Thr	Leu	Glu	Glu	Phe	Gln	Asp	Val	Tyr	Leu	Val	Met	Glu	Leu	Met	Asp
				100					105					110		
	Ala	Asn	Leu	Cys	Gln	Val	Ile	His	Met	Glu	Leu	Asp	His	Glu	Arg	Met
			115					120					125			
35	Ser	Tyr	Leu	Leu	Tyr	Gln	Met	Leu	Cys	Gly	Ile	Lys	His	Leu	His	Ser
		130					135					140				
	Ala	Gly	Ile	Ile	His	Arg	Asp	Leu	Lys	Pro	Ser	Asn	Ile	Val	Val	Lys
	145					150					155					160

	Ser	Asp	Cys	Thr	Leu	Lys	Ile	Leu	Asp	Phe	Gly	Leu	Ala	Arg	Thr	Ala	
					165					170					175		
	Cys	Asn	Phe	Met	Met	Thr	Pro	Tyr	Val	Val	Thr	Arg	Tyr	Tyr	Arg		
			180					185					190				
5	Ala	Pro	Glu	Val	Ile	Leu	Gly	Met	Gly	Tyr	Lys	Glu	Asn	Val	Asp	Ile	
			195					200					205				
	Trp	Ser	Val	Gly	Cys	Ile	Met	Gly	Glu	Leu	Val	Lys	Gly	Cys	Val	Ile	
			210				215						220				
10	Phe	Gln	Gly	Thr	Asp	His	Ile	Asp	Gln	Trp	Asn	Lys	Val	Ile	Glu	Gln	
	225					230					235					240	
	Leu	Gly	Thr	Pro	Ser	Ala	Glu	Phe	Met	Lys	Lys	Leu	Gln	Pro	Thr	Val	
					245					250					255		
	Arg	Asn	Tyr	Val	Glu	Asn	Arg	Pro	Lys	Tyr	Pro	Gly	Ile	Lys	Phe	Glu	
				260					265					270			
15	Glu	Leu	Phe	Pro	Asp	Trp	Ile	Phe	Pro	Ser	Glu	Ser	Glu	Arg	Asp	Lys	
			275					280					285				
	Ile	Lys	Thr	Ser	Gln	Ala	Arg	Asp	Leu	Leu	Ser	Lys	Met	Leu	Val	Ile	
			290				295					300					
20	Asp	Pro	Asp	Lys	Arg	Ile	Ser	Val	Asp	Glu	Ala	Leu	Arg	His	Pro	Tyr	
	305					310					315					320	
	Ile	Thr	Val	Trp	Tyr	Asp	Pro	Ala	Glu	Ala	Glu	Ala	Pro	Pro	Pro	Gln	
					325					330					335		
	Ile	Tyr	Asp	Ala	Gln	Leu	Glu	Glu	Arg	Glu	His	Ala	Ile	Glu	Glu	Trp	
				340					345					350			
25	Lys	Glu	Leu	Ile	Tyr	Lys	Glu	Val	Met	Asp	Trp	Glu	Glu	Arg	Ser	Lys	
			355				360						365				
	Asn	Gly	Val	Val	Lys	Asp	Gln	Pro	Ser	Ala	Gln	Met	Gln	Gln			
		370					375					380					

(2) INFORMATION FOR SEQ ID NO:30:

30 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 382 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Met	Ser	Asp	Ser	Lys	Cys	Asp	Ser	Gln	Phe	Tyr	Ser	Val	Gln	Val	Ala
1				5				10					15		

	Asp	Ser	Thr	Phe	Thr	Val	Leu	Lys	Arg	Tyr	Gln	Gln	Leu	Lys	Pro	Ile
				20					25					30		
	Gly	Ser	Gly	Ala	Gln	Gly	Ile	Val	Cys	Ala	Ala	Phe	Asp	Thr	Val	Leu
			35					40					45			
5	Gly	Ile	Ser	Val	Ala	Val	Lys	Lys	Leu	Ser	Arg	Pro	Phe	Gln	Asn	Gln
		50					55					60				
	Thr	His	Ala	Lys	Arg	Ala	Tyr	Arg	Glu	Leu	Val	Leu	Leu	Lys	Cys	Val
	65					70					75					80
10	Asn	His	Lys	Asn	Ile	Ile	Ser	Leu	Leu	Asn	Val	Phe	Thr	Pro	Gln	Lys
				85						90					95	
	Thr	Leu	Glu	Glu	Phe	Gln	Asp	Val	Tyr	Leu	Val	Met	Glu	Leu	Met	Asp
				100					105					110		
	Ala	Asn	Leu	Cys	Gln	Val	Ile	His	Met	Glu	Leu	Asp	His	Glu	Arg	Met
			115					120					125			
15	Ser	Tyr	Leu	Leu	Tyr	Gln	Met	Leu	Cys	Gly	Ile	Lys	His	Leu	His	Ser
		130					135					140				
	Ala	Gly	Ile	Ile	His	Arg	Asp	Leu	Lys	Pro	Ser	Asn	Ile	Val	Val	Lys
	145					150					155					160
20	Ser	Asp	Cys	Thr	Leu	Lys	Ile	Leu	Asp	Phe	Gly	Leu	Ala	Arg	Thr	Ala
				165						170					175	
	Cys	Thr	Asn	Phe	Met	Met	Thr	Pro	Tyr	Val	Val	Thr	Arg	Tyr	Tyr	Arg
				180					185					190		
	Ala	Pro	Glu	Val	Ile	Leu	Gly	Met	Gly	Tyr	Lys	Glu	Asn	Val	Asp	Ile
			195				200						205			
25	Trp	Ser	Val	Gly	Cys	Ile	Met	Ala	Glu	Met	Val	Leu	His	Lys	Val	Leu
		210					215					220				
	Phe	Pro	Gly	Arg	Asp	Tyr	Ile	Asp	Gln	Trp	Asn	Lys	Val	Ile	Glu	Gln
	225					230					235					240
30	Leu	Gly	Thr	Pro	Ser	Ala	Glu	Phe	Met	Lys	Lys	Leu	Gln	Pro	Thr	Val
				245						250					255	
	Arg	Asn	Tyr	Val	Glu	Asn	Arg	Pro	Lys	Tyr	Pro	Gly	Ile	Lys	Phe	Glu
			260						265					270		
	Glu	Leu	Phe	Pro	Asp	Trp	Ile	Phe	Pro	Ser	Glu	Ser	Glu	Arg	Asp	Lys
			275					280					285			
35	Ile	Lys	Thr	Ser	Gln	Ala	Arg	Asp	Leu	Leu	Ser	Lys	Met	Leu	Val	Ile
		290					295					300				
	Asp	Pro	Asp	Lys	Arg	Ile	Ser	Val	Asp	Glu	Ala	Leu	Arg	His	Pro	Tyr
	305					310					315					320

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Ile Thr Val Trp Tyr Asp Pro Ala Glu Ala Glu Ala Pro Pro Pro Gln
      325                      330                      335

Ile Tyr Asp Ala Gln Leu Glu Glu Arg Glu His Ala Ile Glu Glu Trp
      340                      345                      350

5   Lys Glu Leu Ile Tyr Lys Glu Val Met Asp Trp Glu Glu Arg Ser Lys
      355                      360                      365

Asn Gly Val Val Lys Asp Gln Pro Ser Ala Gln Met Gln Gln
      370                      375                      380

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(2) INFORMATION FOR SEQ ID NO:31:

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10   (i) SEQUENCE CHARACTERISTICS:
      (A) LENGTH: 424 amino acids
      (B) TYPE: amino acid
      (C) STRANDEDNESS: single
      (D) TOPOLOGY: linear

15   (ii) MOLECULE TYPE: protein

      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Met Ser Asp Ser Lys Cys Asp Ser Gln Phe Tyr Ser Val Gln Val Ala
1      5      10      15

Asp Ser Thr Phe Thr Val Leu Lys Arg Tyr Gln Gln Leu Lys Pro Ile
20      20      25      30

Gly Ser Gly Ala Gln Gly Ile Val Cys Ala Ala Phe Asp Thr Val Leu
      35      40      45

Gly Ile Ser Val Ala Val Lys Lys Leu Ser Arg Pro Phe Gln Asn Gln
      50      55      60

25   Thr His Ala Lys Arg Ala Tyr Arg Glu Leu Val Leu Leu Lys Cys Val
      65      70      75      80

Asn His Lys Asn Ile Ile Ser Leu Leu Asn Val Phe Thr Pro Gln Lys
      85      90      95

30   Thr Leu Glu Glu Phe Gln Asp Val Tyr Leu Val Met Glu Leu Met Asp
      100     105     110

Ala Asn Leu Cys Gln Val Ile His Met Glu Leu Asp His Glu Arg Met
      115     120     125

Ser Tyr Leu Leu Tyr Gln Met Leu Cys Gly Ile Lys His Leu His Ser
      130     135     140

35   Ala Gly Ile Ile His Arg Asp Leu Lys Pro Ser Asn Ile Val Val Lys
      145     150     155     160

Ser Asp Cys Thr Leu Lys Ile Leu Asp Phe Gly Leu Ala Arg Thr Ala
      165     170     175

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	Cys	Thr	Asn	Phe	Met	Met	Thr	Pro	Tyr	Val	Val	Thr	Arg	Tyr	Tyr	Arg	
				180					185					190			
	Ala	Pro	Glu	Val	Ile	Leu	Gly	Met	Gly	Tyr	Lys	Glu	Asn	Val	Asp	Ile	
			195					200					205				
5	Trp	Ser	Val	Gly	Cys	Ile	Met	Ala	Glu	Met	Val	Leu	His	Lys	Val	Leu	
		210					215					220					
	Phe	Pro	Gly	Arg	Asp	Tyr	Ile	Asp	Gln	Trp	Asn	Lys	Val	Ile	Glu	Gln	
	225					230					235					240	
10	Leu	Gly	Thr	Pro	Ser	Ala	Glu	Phe	Met	Lys	Lys	Leu	Gln	Pro	Thr	Val	
					245					250					255		
	Arg	Asn	Tyr	Val	Glu	Asn	Arg	Pro	Lys	Tyr	Pro	Gly	Ile	Lys	Phe	Glu	
				260					265					270			
	Glu	Leu	Phe	Pro	Asp	Trp	Ile	Phe	Pro	Ser	Glu	Ser	Glu	Arg	Asp	Lys	
			275					280					285				
15	Ile	Lys	Thr	Ser	Gln	Ala	Arg	Asp	Leu	Leu	Ser	Lys	Met	Leu	Val	Ile	
		290					295					300					
	Asp	Pro	Asp	Lys	Arg	Ile	Ser	Val	Asp	Glu	Ala	Leu	Arg	His	Pro	Tyr	
	305					310					315					320	
20	Ile	Thr	Val	Trp	Tyr	Asp	Pro	Ala	Glu	Ala	Glu	Ala	Pro	Pro	Pro	Gln	
					325					330					335		
	Ile	Tyr	Asp	Ala	Gln	Leu	Glu	Glu	Arg	Glu	His	Ala	Ile	Glu	Glu	Trp	
				340					345					350			
	Lys	Glu	Leu	Ile	Tyr	Lys	Glu	Val	Met	Asp	Trp	Glu	Glu	Arg	Ser	Lys	
			355					360					365				
25	Asn	Gly	Val	Val	Lys	Asp	Gln	Pro	Ser	Asp	Ala	Ala	Val	Ser	Ser	Asn	
		370					375					380					
	Ala	Thr	Pro	Ser	Gln	Ser	Ser	Ser	Ile	Asn	Asp	Ile	Ser	Ser	Met	Ser	
	385					390					395					400	
30	Thr	Glu	Gln	Thr	Leu	Ala	Ser	Asp	Thr	Asp	Ser	Ser	Leu	Asp	Ala	Ser	
					405					410					415		
	Thr	Gly	Pro	Leu	Glu	Gly	Cys	Arg									
					420												

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
- 35 (A) LENGTH: 424 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

	Met	Ser	Asp	Ser	Lys	Cys	Asp	Ser	Gln	Phe	Tyr	Ser	Val	Gln	Val	Ala
	1				5					10					15	
5	Asp	Ser	Thr	Phe	Thr	Val	Leu	Lys	Arg	Tyr	Gln	Gln	Leu	Lys	Pro	Ile
				20					25					30		
	Gly	Ser	Gly	Ala	Gln	Gly	Ile	Val	Cys	Ala	Ala	Phe	Asp	Thr	Val	Leu
			35					40					45			
	Gly	Ile	Asn	Val	Ala	Val	Lys	Lys	Leu	Ser	Arg	Pro	Phe	Gln	Asn	Gln
		50					55					60				
10	Thr	His	Ala	Lys	Arg	Ala	Tyr	Arg	Glu	Leu	Val	Leu	Leu	Lys	Cys	Val
	65					70					75					80
	Asn	His	Lys	Asn	Ile	Ile	Ser	Leu	Leu	Asn	Val	Phe	Thr	Pro	Gln	Lys
					85					90					95	
	Thr	Leu	Glu	Glu	Phe	Gln	Asp	Val	Tyr	Leu	Val	Met	Glu	Leu	Met	Asp
15				100					105					110		
	Ala	Asn	Leu	Cys	Gln	Val	Ile	His	Met	Glu	Leu	Asp	His	Glu	Arg	Met
			115					120					125			
	Ser	Tyr	Leu	Leu	Tyr	Gln	Met	Leu	Cys	Gly	Ile	Lys	His	Leu	His	Ser
		130					135					140				
20	Ala	Gly	Ile	Ile	His	Arg	Asp	Leu	Lys	Pro	Ser	Asn	Ile	Val	Val	Lys
	145					150					155					160
	Ser	Asp	Cys	Thr	Leu	Lys	Ile	Leu	Asp	Phe	Gly	Leu	Ala	Arg	Thr	Ala
					165					170					175	
	Cys	Thr	Asn	Phe	Met	Met	Thr	Pro	Tyr	Val	Val	Thr	Arg	Tyr	Tyr	Arg
25				180					185					190		
	Ala	Pro	Glu	Val	Ile	Leu	Gly	Met	Gly	Tyr	Lys	Glu	Asn	Val	Asp	Ile
			195					200					205			
	Trp	Ser	Val	Gly	Cys	Ile	Met	Gly	Glu	Leu	Val	Lys	Gly	Cys	Val	Ile
		210					215					220				
30	Phe	Gln	Gly	Thr	Asp	His	Ile	Asp	Gln	Trp	Asn	Lys	Val	Ile	Glu	Gln
	225					230					235					240
	Leu	Gly	Thr	Pro	Ser	Ala	Glu	Phe	Met	Lys	Lys	Leu	Gln	Pro	Thr	Val
					245					250					255	
	Arg	Asn	Tyr	Val	Glu	Asn	Arg	Pro	Lys	Tyr	Pro	Gly	Ile	Lys	Phe	Glu
35				260					265					270		
	Glu	Leu	Phe	Pro	Asp	Trp	Ile	Phe	Pro	Ser	Glu	Ser	Glu	Arg	Asp	Lys
			275					280					285			
	Ile	Lys	Thr	Ser	Gln	Ala	Arg	Asp	Leu	Leu	Ser	Lys	Met	Leu	Val	Ile
		290					295					300				

Asp Pro Asp Lys Arg Ile Ser Val Asp Glu Ala Leu Arg His Pro Tyr
305 310 315 320

Ile Thr Val Trp Tyr Asp Pro Ala Glu Ala Glu Ala Pro Pro Pro Gln
325 330 335

5 Ile Tyr Asp Ala Gln Leu Glu Glu Arg Glu His Ala Ile Glu Glu Trp
340 345 350

Lys Glu Leu Ile Tyr Lys Glu Val Met Asp Trp Glu Glu Arg Ser Lys
355 360 365

10 Asn Gly Val Val Lys Asp Gln Pro Pro Asp Ala Ala Val Ser Ser Asn
370 375 380

Ala Thr Pro Ser Gln Ser Ser Ser Ile Asn Asp Ile Ser Ser Met Ser
385 390 395 400

Thr Glu Gln Thr Leu Ala Ser Asp Thr Asp Ser Ser Leu Asp Ala Ser
405 410 415

15 Thr Gly Pro Leu Glu Gly Cys Arg
420

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 384 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

25 Met Ser Arg Ser Lys Arg Asp Asn Asn Phe Tyr Ser Val Glu Ile Gly
1 5 10 15

Asp Ser Thr Phe Thr Val Leu Lys Arg Tyr Gln Asn Leu Lys Pro Ile
20 25 30

30 Gly Ser Gly Ala Gln Gly Ile Val Cys Ala Ala Tyr Asp Ala Ile Leu
35 40 45

Glu Arg Asn Val Ala Ile Lys Lys Leu Ser Arg Pro Phe Gln Asn Gln
50 55 60

Thr His Ala Lys Arg Ala Tyr Arg Glu Leu Val Leu Met Lys Cys Val
65 70 75 80

35 Asn His Lys Asn Ile Ile Gly Leu Leu Asn Val Phe Thr Pro Gln Lys
85 90 95

Ser Leu Glu Glu Phe Gln Asp Val Tyr Ile Val Met Glu Leu Met Asp
100 105 110

	Ala	Asn	Leu	Cys	Gln	Val	Ile	Gln	Met	Glu	Leu	Asp	His	Glu	Arg	Met	
			115					120					125				
	Ser	Tyr	Leu	Leu	Tyr	Gln	Met	Leu	Cys	Gly	Ile	Lys	His	Leu	His	Ser	
			130				135					140					
5	Ala	Gly	Ile	Ile	His	Arg	Asp	Leu	Lys	Pro	Ser	Asn	Ile	Val	Val	Lys	
	145					150					155					160	
	Ser	Asp	Cys	Thr	Leu	Lys	Ile	Leu	Asp	Phe	Gly	Leu	Ala	Arg	Thr	Ala	
				165						170					175		
10	Gly	Thr	Ser	Phe	Met	Met	Thr	Pro	Tyr	Val	Val	Thr	Arg	Tyr	Tyr	Arg	
				180					185					190			
	Ala	Pro	Glu	Val	Ile	Leu	Gly	Met	Gly	Tyr	Lys	Glu	Asn	Val	Asp	Leu	
			195					200					205				
	Trp	Ser	Val	Gly	Cys	Ile	Met	Gly	Glu	Met	Val	Cys	His	Lys	Ile	Leu	
	210					215						220					
15	Phe	Pro	Gly	Arg	Asp	Tyr	Ile	Asp	Gln	Trp	Asn	Lys	Val	Ile	Glu	Gln	
	225					230					235					240	
	Leu	Gly	Thr	Pro	Cys	Pro	Glu	Phe	Met	Lys	Lys	Leu	Gln	Pro	Thr	Val	
					245					250					255		
20	Arg	Thr	Tyr	Val	Glu	Asn	Arg	Pro	Lys	Tyr	Ala	Gly	Tyr	Ser	Phe	Glu	
				260					265					270			
	Lys	Leu	Phe	Pro	Asp	Val	Leu	Phe	Pro	Ala	Asp	Ser	Glu	His	Asn	Lys	
			275					280					285				
	Leu	Lys	Ala	Ser	Gln	Ala	Arg	Asp	Leu	Leu	Ser	Lys	Met	Leu	Val	Ile	
		290					295					300					
25	Asp	Ala	Ser	Lys	Arg	Ile	Ser	Val	Asp	Glu	Ala	Leu	Gln	His	Pro	Tyr	
	305					310					315					320	
	Ile	Asn	Val	Trp	Tyr	Asp	Pro	Ser	Glu	Ala	Glu	Ala	Pro	Pro	Pro	Lys	
					325					330					335		
30	Ile	Pro	Asp	Lys	Gln	Leu	Asp	Glu	Arg	Glu	His	Thr	Ile	Glu	Glu	Trp	
				340					345					350			
	Lys	Glu	Leu	Ile	Tyr	Lys	Glu	Val	Met	Asp	Leu	Glu	Glu	Arg	Thr	Lys	
			355					360					365				
	Asn	Gly	Val	Ile	Arg	Gly	Gln	Pro	Ser	Pro	Leu	Ala	Gln	Val	Gln	Gln	
		370					375					380					

35 (2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 427 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

5	Met 1	Ser	Arg	Ser	Lys 5	Arg	Asp	Asn	Asn	Phe 10	Tyr	Ser	Val	Glu	Ile	Gly 15
	Asp	Ser	Thr	Phe 20	Thr	Val	Leu	Lys	Arg 25	Tyr	Gln	Asn	Leu	Lys 30	Pro	Ile
	Gly	Ser	Gly 35	Ala	Gln	Gly	Ile 40	Val	Cys	Ala	Ala	Tyr	Asp 45	Ala	Ile	Leu
10	Glu 50	Arg	Asn	Val	Ala	Ile	Lys 55	Lys	Leu	Ser	Arg	Pro 60	Phe	Gln	Asn	Gln
	Thr 65	His	Ala	Lys	Arg	Ala 70	Tyr	Arg	Glu	Leu	Val 75	Leu	Met	Lys	Cys	Val 80
15	Asn	His	Lys	Asn 85	Ile	Ile	Gly	Leu	Leu 90	Asn	Val	Phe	Thr	Pro 95	Gln	Lys
	Ser	Leu	Glu	Glu 100	Phe	Gln	Asp	Val	Tyr 105	Ile	Val	Met	Glu	Leu 110	Met	Asp
	Ala	Asn	Leu	Cys 115	Gln	Val	Ile	Gln	Met 120	Glu	Leu	Asp	His 125	Glu	Arg	Met
20	Ser 130	Tyr	Leu	Leu	Tyr	Gln	Met 135	Leu	Cys	Gly	Ile	Lys 140	His	Leu	His	Ser
	Ala 145	Gly	Ile	Ile	His	Arg	Asp 150	Leu	Lys	Pro	Ser	Asn 155	Ile	Val	Val	Lys 160
25	Ser	Asp	Cys	Thr 165	Leu	Lys	Ile	Leu	Asp	Phe 170	Gly	Leu	Ala	Arg	Thr 175	Ala
	Gly	Thr	Ser	Phe 180	Met	Met	Thr	Pro	Tyr 185	Val	Val	Thr	Arg	Tyr 190	Tyr	Arg
	Ala	Pro	Glu	Val 195	Ile	Leu	Gly	Met 200	Gly	Tyr	Lys	Glu	Asn 205	Val	Asp	Leu
30	Trp 210	Ser	Val	Gly	Cys	Ile	Met 215	Gly	Glu	Met	Val	Cys 220	His	Lys	Ile	Leu
	Phe 225	Pro	Gly	Arg	Asp	Tyr 230	Ile	Asp	Gln	Trp	Asn 235	Lys	Val	Ile	Glu	Gln 240
35	Leu	Gly	Thr	Pro 245	Cys	Pro	Glu	Phe	Met	Lys 250	Lys	Leu	Gln	Pro	Thr 255	Val
	Arg	Thr	Tyr	Val 260	Glu	Asn	Arg	Pro	Lys 265	Tyr	Ala	Gly	Tyr	Ser 270	Phe	Glu

	Lys	Leu	Phe	Pro	Asp	Val	Leu	Phe	Pro	Ala	Asp	Ser	Glu	His	Asn	Lys	
				275					280					285			
	Leu	Lys	Ala	Ser	Gln	Ala	Arg	Asp	Leu	Leu	Ser	Lys	Met	Leu	Val	Ile	
		290					295					300					
5	Asp	Ala	Ser	Lys	Arg	Ile	Ser	Val	Asp	Glu	Ala	Leu	Gln	His	Pro	Tyr	
	305					310					315					320	
	Ile	Asn	Val	Trp	Tyr	Asp	Pro	Ser	Glu	Ala	Glu	Ala	Pro	Pro	Pro	Lys	
					325					330					335		
10	Ile	Pro	Asp	Lys	Gln	Leu	Asp	Glu	Arg	Glu	His	Thr	Ile	Glu	Glu	Trp	
				340					345					350			
	Lys	Glu	Leu	Ile	Tyr	Lys	Glu	Val	Met	Asp	Leu	Glu	Glu	Arg	Thr	Lys	
			355					360					365				
	Asn	Gly	Val	Ile	Arg	Gly	Gln	Pro	Ser	Pro	Leu	Gly	Ala	Ala	Val	Ile	
		370					375					380					
15	Asn	Gly	Ser	Gln	His	Pro	Ser	Ser	Ser	Ser	Ser	Val	Asn	Asp	Val	Ser	
	385					390					395					400	
	Ser	Met	Ser	Thr	Asp	Pro	Thr	Leu	Ala	Ser	Asp	Thr	Asp	Ser	Ser	Leu	
					405					410					415		
20	Glu	Ala	Ala	Ala	Gly	Pro	Leu	Gly	Cys	Cys	Arg						
				420				425									

(2) INFORMATION FOR SEQ ID NO:35:

	(i) SEQUENCE CHARACTERISTICS:																
	(A) LENGTH: 384 amino acids																
	(B) TYPE: amino acid																
25	(C) STRANDEDNESS: single																
	(D) TOPOLOGY: linear																
	(ii) MOLECULE TYPE: protein																
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:																
30	Met	Ser	Arg	Ser	Lys	Arg	Asp	Asn	Asn	Phe	Tyr	Ser	Val	Glu	Ile	Gly	
	1				5					10				15			
	Asp	Ser	Thr	Phe	Thr	Val	Leu	Lys	Arg	Tyr	Gln	Asn	Leu	Lys	Pro	Ile	
				20					25					30			
	Gly	Ser	Gly	Ala	Gln	Gly	Ile	Val	Cys	Ala	Ala	Tyr	Asp	Ala	Ile	Leu	
			35				40						45				
35	Glu	Arg	Asn	Val	Ala	Ile	Lys	Lys	Leu	Ser	Arg	Pro	Phe	Gln	Asn	Gln	
		50					55					60					
	Thr	His	Ala	Lys	Arg	Ala	Tyr	Arg	Glu	Leu	Val	Leu	Met	Lys	Cys	Val	
	65					70				75						80	

	Asn	His	Lys	Asn	Ile	Ile	Gly	Leu	Leu	Asn	Val	Phe	Thr	Pro	Gln	Lys
					85					90					95	
	Ser	Leu	Glu	Glu	Phe	Gln	Asp	Val	Tyr	Ile	Val	Met	Glu	Leu	Met	Asp
				100					105					110		
5	Ala	Asn	Leu	Cys	Gln	Val	Ile	Gln	Met	Glu	Leu	Asp	His	Glu	Arg	Met
			115					120					125			
	Ser	Tyr	Leu	Leu	Tyr	Gln	Met	Leu	Cys	Gly	Ile	Lys	His	Leu	His	Ser
		130					135					140				
10	Ala	Gly	Ile	Ile	His	Arg	Asp	Leu	Lys	Pro	Ser	Asn	Ile	Val	Val	Lys
	145					150					155					160
	Ser	Asp	Cys	Thr	Leu	Lys	Ile	Leu	Asp	Phe	Gly	Leu	Ala	Arg	Thr	Ala
					165					170					175	
	Gly	Thr	Ser	Phe	Met	Met	Thr	Pro	Tyr	Val	Val	Thr	Arg	Tyr	Tyr	Arg
				180					185					190		
15	Ala	Pro	Glu	Val	Ile	Leu	Gly	Met	Gly	Tyr	Lys	Glu	Asn	Val	Asp	Ile
			195					200					205			
	Trp	Ser	Val	Gly	Cys	Ile	Met	Gly	Glu	Met	Ile	Lys	Gly	Gly	Val	Leu
		210					215					220				
20	Phe	Pro	Gly	Thr	Asp	His	Ile	Asp	Gln	Trp	Asn	Lys	Val	Ile	Glu	Gln
	225					230					235					240
	Leu	Gly	Thr	Pro	Cys	Pro	Glu	Phe	Met	Lys	Lys	Leu	Gln	Pro	Thr	Val
					245					250					255	
	Arg	Thr	Tyr	Val	Glu	Asn	Arg	Pro	Lys	Tyr	Ala	Gly	Tyr	Ser	Phe	Glu
				260					265					270		
25	Lys	Leu	Phe	Pro	Asp	Val	Leu	Phe	Pro	Ala	Asp	Ser	Glu	His	Asn	Lys
			275					280					285			
	Leu	Lys	Ala	Ser	Gln	Ala	Arg	Asp	Leu	Leu	Ser	Lys	Met	Leu	Val	Ile
		290					295					300				
30	Asp	Ala	Ser	Lys	Arg	Ile	Ser	Val	Asp	Glu	Ala	Leu	Gln	His	Pro	Tyr
	305					310					315					320
	Ile	Asn	Val	Trp	Tyr	Asp	Pro	Ser	Glu	Ala	Glu	Ala	Pro	Pro	Pro	Lys
					325					330					335	
	Ile	Pro	Asp	Lys	Gln	Leu	Asp	Glu	Arg	Glu	His	Thr	Ile	Glu	Glu	Trp
				340					345					350		
35	Lys	Glu	Leu	Ile	Tyr	Lys	Glu	Val	Met	Asp	Leu	Glu	Glu	Arg	Thr	Lys
			355					360					365			
	Asn	Gly	Val	Ile	Arg	Gly	Gln	Pro	Ser	Pro	Leu	Ala	Gln	Val	Gln	Gln
		370					375					380				

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 427 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

10	Met	Ser	Arg	Ser	Lys	Arg	Asp	Asn	Asn	Phe	Tyr	Ser	Val	Glu	Ile	Gly	1	5	10	15
	Asp	Ser	Thr	Phe	Thr	Val	Leu	Lys	Arg	Tyr	Gln	Asn	Leu	Lys	Pro	Ile	20	25	30	
	Gly	Ser	Gly	Ala	Gln	Gly	Ile	Val	Cys	Ala	Ala	Tyr	Asp	Ala	Ile	Leu	35	40	45	
15	Glu	Arg	Asn	Val	Ala	Ile	Lys	Lys	Leu	Ser	Arg	Pro	Phe	Gln	Asn	Gln	50	55	60	
	Thr	His	Ala	Lys	Arg	Ala	Tyr	Arg	Glu	Leu	Val	Leu	Met	Lys	Cys	Val	65	70	75	80
20	Asn	His	Lys	Asn	Ile	Ile	Gly	Leu	Leu	Asn	Val	Phe	Thr	Pro	Gln	Lys	85	90	95	
	Ser	Leu	Glu	Glu	Phe	Gln	Asp	Val	Tyr	Ile	Val	Met	Glu	Leu	Met	Asp	100	105	110	
	Ala	Asn	Leu	Cys	Gln	Val	Ile	Gln	Met	Glu	Leu	Asp	His	Glu	Arg	Met	115	120	125	
25	Ser	Tyr	Leu	Leu	Tyr	Gln	Met	Leu	Cys	Gly	Ile	Lys	His	Leu	His	Ser	130	135	140	
	Ala	Gly	Ile	Ile	His	Arg	Asp	Leu	Lys	Pro	Ser	Asn	Ile	Val	Val	Lys	145	150	155	160
30	Ser	Asp	Cys	Thr	Leu	Lys	Ile	Leu	Asp	Phe	Gly	Leu	Ala	Arg	Thr	Ala	165	170	175	
	Gly	Thr	Ser	Phe	Met	Met	Thr	Pro	Tyr	Val	Val	Thr	Arg	Tyr	Tyr	Arg	180	185	190	
	Ala	Pro	Glu	Val	Ile	Leu	Gly	Met	Gly	Tyr	Lys	Glu	Asn	Val	Asp	Ile	195	200	205	
35	Trp	Ser	Val	Gly	Cys	Ile	Met	Gly	Glu	Met	Ile	Lys	Gly	Gly	Val	Leu	210	215	220	
	Phe	Pro	Gly	Thr	Asp	His	Ile	Asp	Gln	Trp	Asn	Lys	Val	Ile	Glu	Gln	225	230	235	240

```

Leu Gly Thr Pro Cys Pro Glu Phe Met Lys Lys Leu Gln Pro Thr Val
      245                                250                        255

Arg Thr Tyr Val Glu Asn Arg Pro Lys Tyr Ala Gly Tyr Ser Phe Glu
      260                                265                        270

5  Lys Leu Phe Pro Asp Val Leu Phe Pro Ala Asp Ser Glu His Asn Lys
      275                                280                        285

Leu Lys Ala Ser Gln Ala Arg Asp Leu Leu Ser Lys Met Leu Val Ile
      290                                295                        300

10 Asp Ala Ser Lys Arg Ile Ser Val Asp Glu Ala Leu Gln His Pro Tyr
      305                                310                        315                        320

Ile Asn Val Trp Tyr Asp Pro Ser Glu Ala Glu Ala Pro Pro Pro Lys
      325                                330                        335

Ile Pro Asp Lys Gln Leu Asp Glu Arg Glu His Thr Ile Glu Glu Trp
      340                                345                        350

15 Lys Glu Leu Ile Tyr Lys Glu Val Met Asp Leu Glu Glu Arg Thr Lys
      355                                360                        365

Asn Gly Val Ile Arg Gly Gln Pro Ser Pro Leu Gly Ala Ala Val Ile
      370                                375                        380

20 Asn Gly Ser Gln His Pro Ser Ser Ser Ser Ser Val Asn Asp Val Ser
      385                                390                        395                        400

Ser Met Ser Thr Asp Pro Thr Leu Ala Ser Asp Thr Asp Ser Ser Leu
      405                                410                        415

Glu Ala Ala Ala Gly Pro Leu Gly Cys Cys Arg
      420                                425

```

25 (2) INFORMATION FOR SEQ ID NO:37:

```

      (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 364 amino acids
            (B) TYPE: amino acid
            (C) STRANDEDNESS: single
30      (D) TOPOLOGY: linear

```

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```

Met Ser Gly Pro Arg Ala Gly Phe Tyr Arg Gln Glu Leu Asn Lys Thr
1      5      10      15

35 Val Trp Glu Val Pro Gln Arg Leu Gln Gly Leu Arg Pro Val Gly Ser
      20      25      30

Gly Ala Tyr Gly Ser Val Cys Ser Ala Tyr Asp Ala Arg Leu Arg Gln
      35      40      45

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	Lys	Val	Ala	Val	Lys	Lys	Leu	Ser	Arg	Pro	Phe	Gln	Ser	Leu	Ile	His	
	50						55					60					
	Ala	Arg	Arg	Thr	Tyr	Arg	Glu	Leu	Arg	Leu	Leu	Lys	His	Leu	Lys	His	
	65					70				75						80	
5	Glu	Asn	Val	Ile	Gly	Leu	Leu	Asp	Val	Phe	Thr	Pro	Ala	Thr	Ser	Ile	
					85					90					95		
	Glu	Asp	Phe	Ser	Glu	Val	Tyr	Leu	Val	Thr	Thr	Leu	Met	Gly	Ala	Asp	
				100					105					110			
10	Leu	Asn	Asn	Ile	Val	Lys	Cys	Gln	Ala	Leu	Ser	Asp	Glu	His	Val	Gln	
			115					120					125				
	Phe	Leu	Val	Tyr	Gln	Leu	Leu	Arg	Gly	Leu	Lys	Tyr	Ile	His	Ser	Ala	
	130							135					140				
	Gly	Ile	Ile	His	Arg	Asp	Leu	Lys	Pro	Ser	Asn	Val	Ala	Val	Asn	Glu	
	145					150					155					160	
15	Asp	Cys	Glu	Leu	Arg	Ile	Leu	Asp	Phe	Gly	Leu	Ala	Arg	Gln	Ala	Asp	
					165					170					175		
	Glu	Glu	Met	Thr	Gly	Tyr	Val	Ala	Thr	Arg	Trp	Tyr	Arg	Ala	Pro	Glu	
				180					185					190			
20	Ile	Met	Leu	Asn	Trp	Met	His	Tyr	Asn	Gln	Thr	Val	Asp	Ile	Trp	Ser	
			195					200					205				
	Val	Gly	Cys	Ile	Met	Ala	Glu	Leu	Leu	Gln	Gly	Lys	Ala	Leu	Phe	Pro	
		210					215					220					
	Gly	Ser	Asp	Tyr	Ile	Asp	Gln	Leu	Lys	Arg	Ile	Met	Glu	Val	Val	Gly	
	225					230					235					240	
25	Thr	Pro	Ser	Pro	Glu	Val	Leu	Ala	Lys	Ile	Ser	Ser	Glu	His	Ala	Arg	
					245					250					255		
	Thr	Tyr	Ile	Gln	Ser	Leu	Pro	Pro	Met	Pro	Gln	Lys	Asp	Leu	Ser	Ser	
				260					265					270			
30	Ile	Phe	Arg	Gly	Ala	Asn	Pro	Leu	Ala	Ile	Asp	Leu	Leu	Gly	Arg	Met	
			275					280					285				
	Leu	Val	Leu	Asp	Ser	Asp	Gln	Arg	Val	Ser	Ala	Ala	Glu	Ala	Leu	Ala	
		290					295					300					
	His	Ala	Tyr	Phe	Ser	Gln	Tyr	His	Asp	Pro	Glu	Asp	Glu	Pro	Glu	Ala	
	305					310					315					320	
35	Glu	Pro	Tyr	Asp	Glu	Gly	Val	Glu	Ala	Lys	Glu	Arg	Thr	Leu	Glu	Glu	
					325					330					335		
	Trp	Lys	Glu	Leu	Thr	Tyr	Gln	Glu	Val	Leu	Ser	Phe	Lys	Pro	Pro	Glu	
				340					345					350			

Pro Pro Lys Pro Pro Gly Ser Leu Glu Ile Glu Gln
355 360

CLAIMS

We claim:

1. A method for designing an inhibitor of a second serine/threonine kinase or a second tyrosine kinase comprising the steps of:

a. identifying amino acids in an ATP binding site of a first serine/threonine kinase or a first tyrosine kinase which form close contacts with a compound bound to said ATP binding site;

b. employing protein alignment means to identify a second serine/threonine kinase or a second tyrosine kinase that form some, but not all, of the close contacts formed between said compound and said first serine/threonine kinase or said first tyrosine kinase;

c. altering an amino acid in the ATP binding site of said second serine/threonine kinase or said second tyrosine kinase to create a mutant second serine/threonine kinase or a mutant second tyrosine kinase, wherein said compound binds with at least 10-fold greater affinity to said mutant second kinase than to said second kinase;

d. confirming that said compound binds with greater affinity to said mutant second serine/threonine kinase or said mutant second tyrosine kinase than to said second serine/threonine kinase or said second tyrosine kinase; and

e. using molecular modeling means to modify said compound to create an inhibitor of said second kinase, such that said inhibitor binds to said second kinase with at least 10-fold greater affinity than said compound binds to said second kinase.

7. The method according to claim 6, wherein

a. when said second kinase is ERK2, said mutant second kinase is an ERK-2 mutant having an amino acid sequence as set forth in SEQ ID NO:2, wherein amino

acid 105 is threonine or alanine; or

b. wherein said second kinase is JNK3, said mutant second kinase is a JNK3 mutant comprising amino acids 40-402 of SEQ ID NO:3, wherein amino acid 146 is alanine or threonine.

8. The method according to claim 7, wherein in SEQ ID NO:2 amino acid 103 is leucine, amino acid 106 is histidine, amino acid 109 is glycine and amino acid 110 is alanine.

9. The method according to claim 7, wherein in SEQ ID NO:3 amino acid 150 is glycine.

10. A mutant second serine/threonine kinase or tyrosine kinase characterized by:

a. at least one amino acid substitution in an ATP binding site as compared to a corresponding naturally occurring second kinase;

b. the ability to bind with a K_i or a K_d of less than 10 μ M a compound that binds to an ATP binding site of a first serine/threonine kinase or tyrosine kinase; and

c. the ability to bind said compound with at least a 10-fold lower K_i or K_d than the K_i or K_d for said compound with said second kinase.

11. The mutant second kinase according to claim 10, wherein said first and said second kinases are MAP kinases.

12. The mutant second kinase according to claim 11, wherein said mutant second kinase is selected

from:

a. a mutant ERK2 consisting of the amino acid sequence as set forth in SEQ ID NO:2, wherein amino acid 105 is threonine or alanine; or

b. a mutant JNK3 comprising amino acids 40-402 of SEQ ID NO:3, wherein amino acid 146 is threonine or alanine.

13. The mutant second kinase according to claim 12, wherein in SEQ ID NO:2 amino acid 103 is leucine, amino acid 106 is histidine, amino acid 109 is glycine and amino acid 110 is alanine.

14. The mutant second kinase according to claim 12, wherein in SEQ ID NO:3 amino acid 150 is glycine.

15. A crystallizable co-complex of a mutant second kinase according to any of claims 10 to 14 and an inhibitor of said first kinase bound to the ATP binding site of said mutant second kinase.

16. The crystallizable co-complex according to claim 13, wherein said first kinase is p38, said second kinase is a MAP kinase and said inhibitor is a pyridinyl-imidazole inhibitor of p38.

17. The co-complex according to claim 13, wherein said pyridinyl-imidazole inhibitor of p38 is selected from SB203580 or SB202190.

ABSTRACT

The invention relates to methods for designing inhibitors of serine/threonine kinases and tyrosine kinases, particularly MAP kinases, through the use of
5 ATP-binding site mutants of those kinases. The methods of this invention take advantage of the fact that the mutant kinases are capable of binding inhibitory compounds of other kinases with greater affinity than the corresponding wild-type kinase. The invention further
10 relates to the mutant kinases themselves and crystallizable co-complexes of the mutant kinase and the inhibitory compound.

VPI/97-104 CON

DECLARATION AND POWER OF ATTORNEY
FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled: METHODS FOR DESIGNING INHIBITORS OF SERINE/THREONINE KINASES AND TYROSINE KINASES

the specification of which

(check ☒ [X] is attached hereto one)

[] was filed on _____ as
Application Serial No. _____
and was amended on _____.
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I do not know and do not believe that the invention was ever patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to this application.

I do not know and do not believe that the invention was in public use or on sale in the United States of America more than one year prior to this application.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known by me to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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